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THE SIGNIFICANCE OF SOME SEXUAL PHENOMENA IN THE FUNGI

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INTRODUCTION

The fungi are remarkable for the diversity shown in the manifestation of sex. This has led in the past to much controversy and confusion, some of which still exists. It appears to have arisen from a universal uncertainty as to the significance of sexual reproduction for living organisms, and the essential criteria of sex. However, there is now fairly general agreement on these points, which has made possible a fuller understanding of some of the phenomena of sex in the fungi. Nevertheless, it will be instructive to examine first the earlier views on the subject.

In all the early studies of fungi, the essential feature of sex was taken to be the fusion of male and female gametes or gametangia. Where the gametes or gametangia are all alike, the condition was regarded as a lower grade of sexuality, and consequently less beneficial to the species. This point of view may be illustrated by the following quotations: De Bary (1884, English translation 1887, p. 233) wrote: "To understand clearly this much discussed question we must first of all remember that, in our imperfect knowledge of the nature of sexuality and the sexual process of fertilisation, we have no simple mark or reagent by which we can recognise the sexual quality of an organ. We learn from the facts before us that in every process of fertilisation there is a material union of one peculiar male or fertilising cell or at least of a portion of its protoplasmic and nuclear substance with one other, a female cell, which is to be fertilised. — —The result of this union is that the female portion is rendered capable of further development: the development does not take place without this union, and union with the male portion is necessary that the female may become capable of it. In a doubtful case therefore the determination will depend first on the observation of the union of the protoplasm or nucleus, and secondly on the experimental proof of the necessity of this in order that the presumed female portion may become capable of development." Arthur (1929, p. 77) wrote: "Some form of sexuality appears to be universal, or nearly so, among all classes of organisms, whether animal or plant. In speaking of sexuality in this general way we may define it to

consist of union of two distinct and usually more or less distantly related masses of protoplasm, with their nuclei, chromosomes and whatever else constitutes the energizing factors of the living cell, followed by a constitutional reorganization.... In the highest expression of the sexual process the contents of the male element, or at least the nucleus, passes over into the (generally much larger) female element, where the two nuclei fuse at once or after the lapse of a period during which more or less growth intervenes."

With the recognition of the nuclear cycle (the doubling of chromosome number at nuclear fusion and its halving at meiosis), the emphasis was transferred from the fusing organs to the fusing nuclei. Thus Lotsy 1907, p. 424 (quoted by Arthur 1929) wrote : "The essence of the sexual process does not lie in the union of two sex organs, but in the union of two nuclei." However, the truth of this statement does not appear to have been generally accepted until recently. Thus Gwynne-Vaughan and Barnes (1930, p. 4) give the erroneous impression that absence of male and female organs in most higher fungi signifies an absence of sexual reproduction : "The replacement of normal sexual fusion by the union of two female or two vegetative nuclei, or of a female and a vegetative nucleus is common among fungi, and the complete disappearance of a sexual process is still more frequent. It has been suggested that the variety of food material which fungi, as parasites or saprophytes, obtain from their substratum, may make the stimulus of fertilisation less important ; it is also possible that among these plants competition is less severe than among holophyta or holozoa, and that, being already well adapted to a fairly constant environment, they would not benefit by the chances of variation involved in sexual reproduction. At any rate almost every group of fungi shows a progressive disappearane of normal sexuality."

THE SIGNIFICANCE OF SEXUAL REPRODUCTION

It is now generally accepted that the primary function of sexual reproduction lies in the recombination of hereditary differences between different individuals of a species, with consequent greatly increased adaptability (Fisher 1930, Muller 1932, Mather 1940). The regular alternation of nuclear fusion and meiosis in the life-cycle, coupled with devices to secure the fusion of nuclei from different individuals, are now seen to be the fundamental features of sex, while the presence of morphologically differentiatated "sex organs" to which the terms male and female can be applied is of secondary importance. Thus the emphasis has changed entirely, from organs associated with protoplasmic fusion to a cycle of nuclear events in the course of the life-history. It is advisable to draw a clear distinction, as Darlington (1937, p. 18) has done, between sexual reproduction, defined as nuclear fusion and meiosis in the life-cycle, and sexual differentiation, defined as the presence of morphologically-differentiated structures associated with sexual fusion, i. e. gametes or gametangia. The former is an essential criterion of sexual reproduction, while the latter is not. In most groups of organisms sexual reproduction is always associated with sexual differentiation, and then there is not the same necessity to draw a clear distinction between them. In the fungi, however, this distinction is important, since most of the higher fungi show nuclear fusion and meiosis in the life-cycle, despite the absence of gametangia.

THE HYPOTHESIS OF TWO NUCLEAR FUSIONS AND TWO REDUCTIONS OF CHROMOSOME NUMBER IN THE LIFE-HISTORY OF THE ASCOMYCETES

The occurrence of nuclear fusion in the young ascus of Ascomycetes, first described by Dangeard (1894) for *Aleuria vesiculosa* (Bull. et Fr.) Boud. (syn. *Peziza vesiculosa* Bull.), has been confirmed for numerous species. Harper (1895) claimed that in *Sphaerotheca humuli* (DC.) Burr. a second nuclear fusion occurs in the ascogonium, prior to the formation of asci, and similar claims have subsequently been made for a number of species (See Gwynne-Vaughan & Barnes 1937). There is general agreement that the first two nuclear divisions in the ascus, following the nuclear fusion, constitute meiosis. Those who have claimed that there are two nuclear fusions in the life-history of some Ascomycetes, maintain that, at the third nuclear division in the ascus the chromosome number is halved a second time by a process called ' brachymeiosis '.

There are numerous reasons for believing that the observations of Harper and his followers were in error, and that in fact in the life-history of the Ascomycetes there occurs only one nuclear fusion (in the young ascus) and one reduction of chromosome number (meiosis–the first two nuclear divisions in the ascus). Thus :

(1) Whenever genetical studies have been made on Ascomycetes, they have invariably supported the hypothesis of a single nuclear fusion and a single meiosis in the life-history, and contradicted the theory of two fusions and two reductions.

(2) The chromosomes of fungi are very small and often difficult to stain satisfactorily. This renders counts of chromosome numbers unreliable.

(3) If two nuclear fusions occur in the life-cycle, the fusion nucleus in the young ascus would be polyploid. Meiosis in polyploids shows characteristic features, which have not been observed in Ascomycetes.

(4) Meiosis is known to consist of one chromosome cycle of division superimposed on two nuclear divisions. Both nuclear divisions are thus integral parts of the process. If a second reduction of chromosome number occurs in the Ascomycetes, two additional nuclear divisions would be required, whereas in fact only one is usually found.

(5) Recombination of hereditary differences is brought about by a single nuclear fusion and a single meiosis in the life-history. Two nuclear fusions followed by two chromosomal reductions would appear to be of no further advantage to a species in this respect.

(6) At the time of Harper's original claim, the place of nuclear fusion in the life-histories of many organisms was still unknown, and he was perhaps influenced by the supposedly universal occurrence (with the exception of apomictic organisms) of nuclear fusion immediately after fusion of the gametes or gametangia.

(7) A delay in fusion, following the association of the male and female nuclei, has a selective advantage. It allows the paired nuclei to proliferate many times, so that instead of one nuclear fusion followed

by one meiosis, as in many Phycomycetes, there is a nuclear fusion and meiosis in each of the numerous asci resulting from the association of a single original pair of nuclei. The four primary products of meiosis will in general differ in their genetic constitution both from one another and from the products of other meioses. Hence proliferation prior to meiosis increases the number of genotypes formed, whereas proliferation after meiosis, as in the Mucorales, does not. Genotypes favourable to the environment of the species are more likely to appear when there is an increase in the number of different genotypes formed, and hence adaptation will be promoted.

Despite this weight of evidence against the hypothesis of two nuclear fusions and two chromosome reductions in the Ascomycetes, considerable emphasis is given to it by some authors, e.g. Gwynne-Vaughan and Barnes (1937). One of the most recent claims of its supposed occurrence has been that of Foster (1938, 1941) for *Phaeobulgaria inquinans* (Fr.) Nannf. [syn. *Bulgaria inquinans* (Pers.) Fr.]. He made cytological studies of this fungus and maintained that, despite the absence of recognisable sex organs (gametangia), double nuclear fusion followed by brachymeiosis, occurred regularly in its life-history. This fungus has asci containing 4 brown spores and 4 smaller colourless spores. Whitehouse (1947) has shown that the frequencies of the various arrangements of the spores in the ascus are such as would be expected with (a) normal segregation at meiosis of a pair of allelomorphic genes controlling the appearance of the spores, followed by (b) mitosis and associated with (c) a limited amount of relative displacement of nuclei through the over-lapping of the meiotic and mitotic spindles prior to the development of an ascospore wall round each nucleus. This contradicts the hypothesis of double fusion and brachymeiosis, and Foster's claim, based solely on cytological evidence, is not confirmed genetically.

Until reliable evidence, such as that provided by genetical data is obtained in its support, the hypothesis of two nuclear fusions and two chromosome reductions in the life-history of the Ascomycetes is best regarded as not substantiated.

THE DISAPPEARANCE OF MALE AND FEMALE ORGANS IN THE EVOLUTION OF THE HIGHER FUNGI

On the older view of the nature of sexual reproduction, fungi in which male and female structures were present but failed to undergo fusion, or in which such structures were absent, were regarded as sexually degenerate, or asexual. On the modern view, such organisms are regarded as not necessarily sexually degenerate, provided that nuclear fusion and meiosis occur in the life-cycle. Indeed, a fungus showing a degenerate form of sexuality would nowadays be regarded as one in which self-fertilisation occurs regularly, irrespective of the presence or not of morphologically differentiated "sex organs". Thus, any monoecious or homothallic fungus in which fertilisation occurs by the fusion of gametangia or of sister nuclei is likely to be regularly self-fertilised, and hence may be regarded as sexually degenerate, since the vital recombination of hereditary differences is lost in the absence of crossing between different strains. The Hymenomycetes and

Gasteromycetes, in which " sex organs " (in the original sense of gametes and gametangia) are absent, are not asexual or even sexually degenerate. In fact, the heterothallism, which they possess is of a type such as to give more efficient outbreeding than in any other fungi (Mather 1942, Whitehouse 1949 a, b). Thus, ironically, the only groups of fungi which were formerly thought to show no trace of sexuality, have the most efficient type of sexual reproduction known in the fungi, or indeed in any heploid organism (see Whitehouse 1950).

The type of heterothallism found in many isogamous Algae such as species of *Chlamydomonas*, *Ulva* and *Ectocarpus* may be regarded as essentially a difference of sex in which there is physiological but no morphological differentiation of the sexes. The condition of heterothallic species of Mucorales can perhaps be regarded as similar. But the heterothallism of the Ascomycetes cannot be so regarded, at least in such species as *Ascobolus magnificus* Dodge in which antheridia and ascogonia are present. It is likely that heterothallism in these fungi has evolved secondarily through selection favouring outbreeding in species in which the presence of both male and female organs on the same mycelium had previously led to inbreeding (see Fig. 1). It is possible that some oogamous Algae have become heterothallic secondarily in a similar way.

The presence of non-functional antheridia and oogonia (ascogonia) in some Ascomycetes, and the total absence of such organs in other Ascomycetes and in all the Hymenomycetes and Gasteromycetes, suggest that male and female structures were formerly present and fully functional in the ancestors of all these fungi, but have gradually lost their function and eventually disappeared in the course of evolution. No phenomenon comparable to this is known in the Algae or indeed in any other group of organisms. The old idea that these species were analogous to apogamous ferns and apomictic flowering plants can be dismissed, since it is now known that although the male and female organs in these fungi have lost their function or even disappeared, the nuclear fusion and meiosis remain.

An explanation of the loss of function and ultimate disappearance of the male and female organs during the course of evolution can be found in the phenomenon of heterokaryosis (See Whitehouse 1949 b). Fungi normally show frequent fusion of hyphae in the soil or host in which they grow. Such anastomosis, when between hyphae derived from different spores, will usually result in a heterokaryotic mycelium and is then likely to increase the vigour of growth. This will occur if deleterious recessive genes are present in the nuclei of either strain, provided the same genes are not also present in the other strain. This hyphal anastomosis owing to the heterokaryosis that frequently results from it, is likely to be favoured by natural selection. In this way, unlike nuclei derived from different individuals of the species, will be brought into a common mycelium at an early stage of development. For efficient sexual reproduction, the fusion of unlike nuclei is essential, and it is therefore not surprising, once heterokaryosis has become established in a species, that selection favours the presence of unlike nuclei in the mycelium, not merely as a means of suppressing the effects of deleterious recessive genes, but also as a means of bringing about recombination of hereditary differences through nuclear fusion and meiosis. Thus the anastomoses,

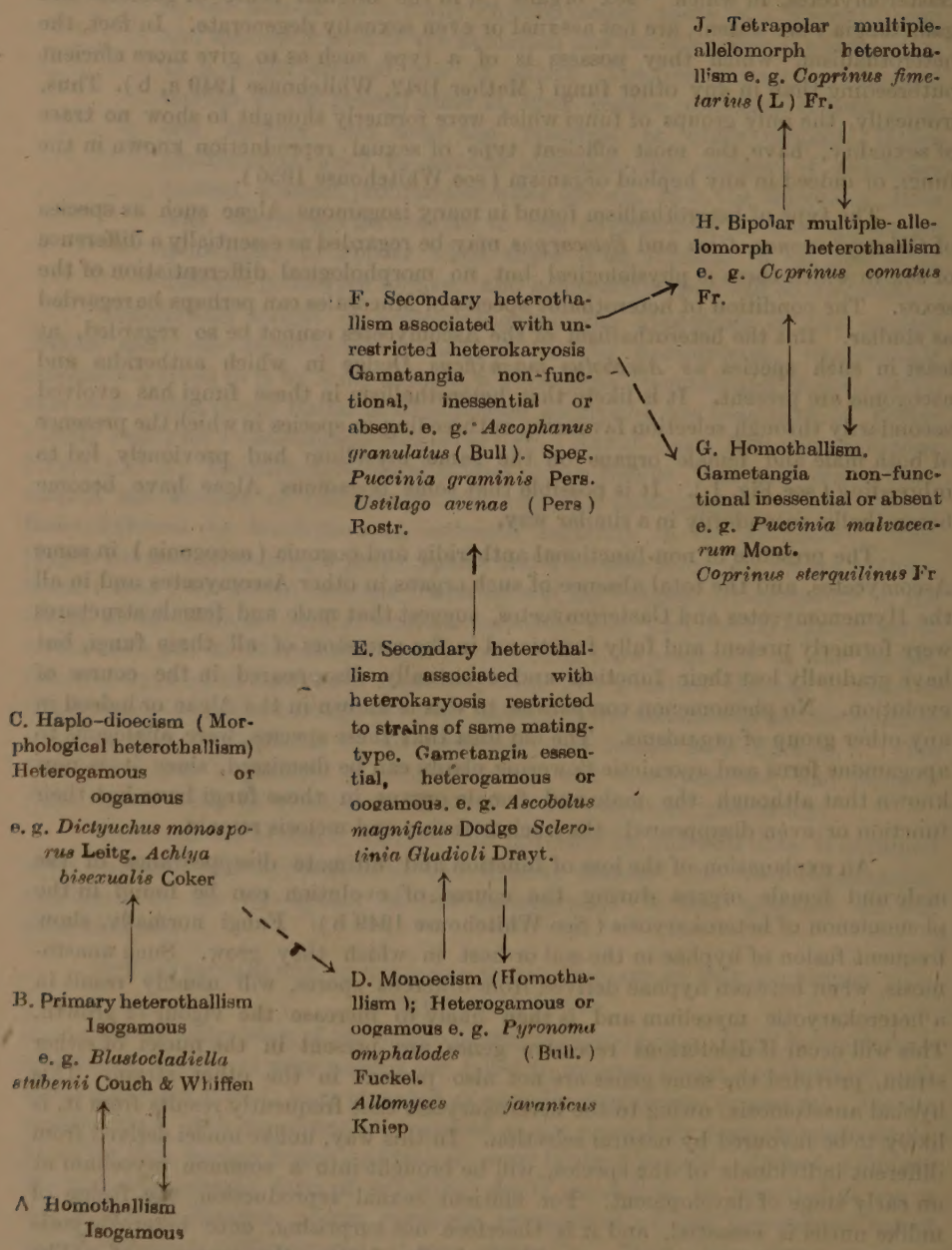

FIG. 1 SCHEME OF PROBABLE EVOLUTION OF SEXUAL PHENOMENA IN THE FUNGI

Broken arrows indicate downgrade changes, associated with increased inbreeding. The position of the heterothallic Mucorales in the scheme is uncertain : they may belong either to B. or E. (In the latter the gametangia would be secondarily isogamous).

which were originally vegetative, take on a sexual function and thus replace that of the gametes or gametangia. The life-history of *Ascophanus granulatus* (Bull.) Speg. [syn. *Humaria granulata* Quel.] is particularly instructive in this respect. It is heterothallic and has ascogonia but no antheridia. Fusion of vegetative hyphae of the 'plus' and 'minus' strains occurs, and the pairs of unlike nuclei migrate to the ascogonia and ultimately fuse in the young asci. (Gwynne-Vaughan and Williamson 1930).

Thus an explanation for the loss during the course of evolution of the function of the gametangia in bringing together unlike nuclei, followed by disappearance of the gametangia themselves, is to be found in the phenomena of heterothallism and heterokaryosis. The probable steps in this process may be summarised as follows:—

(1) Haplodioecious fungus, with male and female organs borne on different strains, becomes monoecious on account of the inevitable short-term advantage of proximity of male and female organs giving greater fertility. (C to D in Fig. 1).

(2) Physiological heterothallism appears, favoured by selection when the long-term disadvantages of inbreeding (and the consequent lack of variability and hence of adaptability) become evident. (D to E in Fig. 1).

(3) Nuclei of opposite mating-types are brought together through vegetative fusion of hyphae, prior to the formation of the male and female organs (F in Fig. 1). In the Ascomycetes, the nuclei which associate in an ascogonium do not immediately fuse, but divide and migrate in pairs into the ascogenous hyphae. It is probable that this condition is favourable for the transference of sexual function from the male and female organs to vegetative hyphae. In the Phycomycetes, there is no replacement of the function of the gametangia by vegetative hyphae, and this may be because the gametangia act as the place of nuclear fusion and of the formation of a thick-walled resting-spore.

(4) The gametangia are now functionless, and may be expected gradually to disappear in the course of evolution. The male organ in likely to disappear first, since the female forms an integral part of the developmental sequence leading to ascogenous hyphae and asci.

With the replacement of the male and female organs by vegetative association of unlike nuclei, the efficiency of sexual reproduction as a means of securing recombination is not diminished, provided opportunity exists (a) for transfer of nuclei within the population of the species and (b) for nuclear fusion to occur between unlike nuclei. The dispersal of spores usually by wind, allows those from different sources to germinate in proximity, while the existence of heterothallism prevents the fusion of identical nuclei. In many of the higher fungi, devices exist which reduce the risk of failure of sexual reproduction, due to failure of association of nuclei of different mating-types. In the Hymenomycetes

and Gasteromycetes, oidia are frequently produced from hyphal tips of strains of a single mating-type, but are only rarely formed once nuclei of compatible mating types have become associated (Brodie 1931). The primary function of the oidia is apparently concerned with the association of strains of unlike mating-type, though oidia are nevertheless capable of germinating on any suitable substratum, and hence readily propagate the strain vegetatively. For this reason, they are often regarded as asexual, though their primary function appears to be sexual. In the Uredinales, the spermatia have a corresponding function, but are incapable of developing except when they come in contact with hyphal tips of the opposite mating-type. Their sexual function is thus more apparent. The oidia of the Hymenomycetes and Gasteromycetes and the spermatia of the Uredinales are apparently not essential for associating unlike nuclei, since this association can be brought about by vegetative fusion of hyphae of compatible mating-types. This has two consequences: (1) The occurrence of a structure to which the term 'female' is applicable need not be expected, since a vegetative hypha is adequate for the reception of spermatium or oidium. (2) Since the spermatia and oidia appear to be adaptations which favour the association of strains of different mating-types, they may have evolved secondarily, following the replacement of fusion of gametangia by fusion of vegetative hyphae. The spermatia and oidia are thus not necessarily homologous with the male organs presumed to have existed in the ancestors of these fungi. If the spermatia and oidia played an essential part in the life-history, such that nuclei of compatible mating-types could not become associated by any other means, then they would probably be homologous with similar organs in those Ascomycetes in which "sex organs" are essential for associating the nuclei which will ultimately fuse.

THE RELATIONSHIP BETWEEN THE USTILAGINALES, UREDINALES, HYMENOMYCETES AND GASTEROMYCETES

There is wide divergence of opinion at the present time concerning the relationship between these groups of fungi. In one of the earliest classifications of the fungi, Fries (1832) recognised the Hymenomycetes and Gasteromycetes as separate classes of fungi, while the Ustilaginales and Uredinales were placed together in a sub-class Hypodermii of a third class, the Coniomycetes. The remaining members of the Coniomycetes are now placed in the Fungi Imperfecti. De Bary (1866) introduced the term Basidiomycetes for the Hymenomycetes and Gasteromycetes and he raised Fries' subclass Hypodermii to the rank of a class. Subsequently (de Bary 1884) he separated the Ustilaginales and Uredinales from one another, associating the Ustilaginales with Protomyces, since both show a sexual fusion of hyphae shortly after germination of the spores, and placing them between the Phycomycetes and Ascomycetes, while the Uredinales he regarded as forming a connecting link between the Ascomycetes and the Basidiomycetes, but with their closest affinities with such Ascomycetes as *Collema* and *Polysigma*.

Brefeld (1888), as a result of cytological studies, subdivided De Bary's class Basidiomycetes into those with a septate basidium, which he called Protobasidiomycetes (the subdivision Tremellales of the Hymenomycetes) and

those with a non-septate basidium, the Autobasidiomycetes (corresponding to the subdivision Agaricales of the Hymenomycetes together with the Gasteromycetes). The similarity between the basidium of the Tremellales and the promycelium of the Uredinales, first pointed out by Tulasne (1853), led Brefeld to suggest that these two groups might have arisen from a common ancestor, and by his use of the term Protobasidiomycetes he implied that he regarded the Tremellales as the most primitive Basidiomycetes. The following year (Brefeld 1889) he went a step further and proposed that the Uredinales should be incorporated in the Basidiomycetes, and extended his subdivision Protobasidiomycetes to include them. The Ustilaginales were omitted from this class and described as Hemibasidii, but Van Tieghem (1893) included them in the Basidiomycetes. At about this time the existence of a cycle of nuclear fusion and meiosis in these fungi became known, and it was realised that the thick-walled resting-spore and the promycedium of the Ustilaginales aud Uredinales corresponded in this respect with the basidium of the Hymenomycetes and Gasteromycetes, supporting the hypothesis of a close relationship between these groups of fungi. It was not surprising, therefore, that the proposal of Brefeld and Van Tieghem to incorporate the Ustilaginales and Uredinales in De Bary's class Basidiomycetes was widely accepted.

Van Tieghem carried the system of classifying primarily by the characters of the basidium and promycelium to its logical conclusion, when he recognized two subdivisions : (1) Phragmobasidia, with septate basidia or promycelia, in which he placed the Tremellales, the Uredinales and the family Ustillaginaceae of Ustilaginales, and (2) Holobasidia, with non-septate basidia or promycelia, in which he included Agaricales, Gasteromycetes and the family Tilletiaceae of Ustilaginales. This system of classification was clearly not satisfactory, since the undoubtedly natural group of the Ustilaginales was split. Patouillard (1900) introduced yet another system, using the terms Homobasidiae and Heterobasidiae. The Homobasidiae corresponded to Brefeld's Autobasidiomycetes, while the Heterobasidiae comprised the Tremellales, Uredinales and Ustilaginales, the latter two sub-classes being relegated to tribes within the family Auriculariaceae. The systems of Brefeld and of Patouillard have subsequently been widely used. In both, the presenee or absence of cross-walls in the basidium or promycelium is regarded as of primary importance, though the fact that within the Ustilaginales some species have a septate promycelium and some non-septate is overlooked.

Despite a widespread acceptance of these systems of classification, it is generally conceded that the Uredinales form one of the most natural and distinctive groups of fungi, and that the Ustilaginales also form a well-defined group. Similarly, the Hymenomycetes and Gasteromycetes are frequently recognized as collectively forming a natural alliance, often described as the " True Basidiomycetes ", the " Larger Basidiomycetes" or the "Higher Basidiomycetes". A few botanists have been unwilling to accept the inclusion by Brefeld and Van Tieghem of the Ustilaginales and Uredinales within the Basidiomycetes. Thus Church (1919, pp. 58-59) wrote : " 'The so-called Brefeldian System' of Hemibasidii and Protobasidiomycetes may be wholly rejected as non-illuminative and serving no useful function beyond mere classification; but rather tending to

obscure fundamental factors. To consider, for example, the massive perennial growth of Polypori, 2–3 feet in diameter, or free organism living wholly in soil (*Agaricus*, *Lycoperdon*), as ever evolved from minute holoparasites of green foilage–leaves of the type of the Uredineae or even *Exobasidium*, from mere details of the little–understood basidium, whether septate or non–septate, is beyond serious discussion" Again, Church (p. 61) wrote : "The Basidiomycete" (in De Bary's sense) "..... is significant as affording a view of the mechanism of initiation of conidial extensions from the tetrad units, very much in the manner of the production of sporidia from a germinating teleutospore of the Uredine; but clearly in an entirely distinct phylum, and so far biologically convergent as the expression of adaptation to similar conditions of environment, and the demand for air–borne spores with a minimum of trouble in production." Others who have maintained this point of view, namely, that the Ustilaginales and Uredinales are so distinct from the Hymenomycetes and Gasteromycetes as not to be included in the Basidiomycetes, are Clements and Shear (1931), who have proposed the term Promycetes for the Ustilaginales and Uredinales, and Bessey (1935), who has suggested the term Teliosporeae. More recently, Bessey (1950) has "with considerable reluctance" reduced the Teliosporeae to the position of a sub–class within the class Basidiomycetes.

It is interesting to examine evidence of the relationship between the Ustilaginales, Uredinales, Hymenomycetes and Gasteromycetes in the light of modern views of the significance of sexual reproduction. In all these groups of fungi, nuclei of compatible mating–types can become associated by fusion of undifferentiated hyphae, and the gametangia where these are present, are not essential for associating unlike nuclei. As indicated in section 4, this condition of unrestricted heterokaryosis is with little doubt an advanced character, as compared with the condition in some of the Ascomycetes, where heterokaryosis is restricted to within strains of the same mating-type, and the gametangia are essential for bringing together the unlike nuclei. Thus it is probable that the Ustilaginales, Uredinales, Hymenomycetes and Gasteromycetes have all been derived from ancestors of Ascomycete type. The homology of the clamp-connection with the ascogenous hook, originally suggested by Kniep (1915), is thus put on a more secure foundation. Moreover this is supported by (a) the presence of clamp–connections in the ascogenous hyphae of species of the genus *Tuber*, and (b) the finding by Greis (1938) that the mode of origin of the ascogenous hook in *Tuber* is essentially like the development of a clamp-connection. If the Ustilaginales and Uredinales have been derived from ancestors of Ascomycete type, then the ascus must be homologous with the thick-walled resting-spore (chlamydospore in Ustilaginales, teleutospore in Uredinales) together with the promycelium which develops from it. The non-septate promycelium found in the family Tilletiaceae of the Ustilaginales would thus appear to be more primitive than the septate promycelium of the family Ustilaginaceae and of the Uredinales. Similarly if the Hymenomycetes and Gasteromycetes have been derived from ancestors of Ascomycete type, then the ascus and basidium are homologous structures, as suggested by Kniep (1915,

1916). This implies that the non-septate basidium is likely to be more primitive than the septate basidium. As Church (1919) states, "the septate basidium does not occur in any type with special claim to be regarded as primitive." Indeed, the idea that the Tuberales and Hymenogastrales may form a connecting link between the Ascomycetes and the Gasteromycetes as suggested by Holm (1949), is more plausible than Brefeld's view that the Auriculariaceae are primitive. The hypothesis that the Hymenomycetes have been derived from the Uredinales via the Auriculariaceae, though still popular (see for instance, Wilson 1950), has little evidence in its favour. As Church (1919) pointed out in the passage already quoted, it is most unlikely that small specialised leaf-parasites such as the Uredinales should have given rise in evolution to the large saprophytic forms characteristic of the Hymenomycetes. Moreover, the widespread occurrence of clamp-connections in the Hymenomycetes and Gasteromycetes associates these groups directly with the Ascomycetes, rather than with the Ustilaginales and Uredinales where clamp-connections occur only rarely.

The converse hypothesis, deriving the Uredinales from the Hymenomycetes through the Auriculariaceae, is also unlikely to be correct. The Ustilaginales and Uredinales have been found to possess the simple Ascomycete type of heterothallism with two strains, often called ' plus ' and ' minus ' (see Whitehouse 1949 b, 1951). On the other hand, the Hymenomycetes (including *Auricularia* and other Tremellales) and the Gasteromycetes have a more complex type in which multiple strains occur (see Whitehouse 1949 a). It is probable that these two types of heterothallism differ rather fundamentally in their physiological mechanism. The one demands the presence of specific strains, while in the other, fusion of any two compatible strains out of a total of perhaps a hundred can lead to sexual reproduction. It is significant that the multiple-strain type of heterothallism is more efficient as an outbreeding mechanism than that involving only two strains (Mather 1942), and it is unlikely, therefore, that the Ustilaginales and Uredinales, with their less efficient type of heterothallism, have been derived from the Hymenomycetes and Gasteromycetes.

The presence of two-allelomorph heterothallism in the Ustilaginales and Uredinales and multiple-allelomorph heterothallism in the Hymenomycetes and Gasteromycetes suggests that the Ustilaginales and Uredinales are more closely related to one another than to the Hymenomycetes and Gasteromycetes, and that the latter two groups are themselves closely related. Bessey (1950, p 644) has listed a number of other characters which tend to associate the Ustilaginales and Uredinales. The most important of these are the intercellular parasitism of vascular plants and the production of thick-walled resting-spores (chlamydospores or teleutospores) and promycelia, in which nuclear fusion and meiosis take place. The exclusive occurrence of multiple-allelomorph heterothallism in the Hymenomycetes and Gasteromycetes and the absence of two-allelomorph heterothallism suggest a monophyletic origin for these fungi. It thus seems likely that the Ustilaginales and Uredinales on the one hand and the Hymenomycetes and Gasteromycetes on the other separated from one another early in their evolutionary histories. The main features which they have in common and

which are not possessed by their presumed ancestors of Ascomycete type, are the external production of the post-meiotic spores and their abstriction by a drop mechanism. These features perhaps arose prior to the divergence of the groups, but their common ancestry presumably ended before the development of specialised parasitism and a thick-walled resting-spore (chlamydospore or teleutospore) in the Ustilaginales and Uredinales, and before the devlopment of multiple-strain heterothallism in the Hymenomycetes and Gasteromycetes. It is evident that Brefeld, Van Tieghem and Patouillard attached undue importance to the septation of the promycelium and the basidium, and that, as Church (1919) pointed out, this emphasis on a trivial character has tended to obscure the true relationships of these groups of fungi.

I suggest therefore that the original use of the term Basidiomycetes for the Hymenomycetes and Gasteromycetes (De Bary 1866, 1884) be revived and that the Ustilaginales and Uredinales together be recognized as a separate class of the fungi. This will make necessary a return to the use of the terms 'promycelium' and 'sporidium' in place of 'basidium' and 'basidiospore' respectively, when referring to the Ustilaginales and Uredinales. Terms that have been adopted at various times for the latter two orders of fungi are Hypodermii (Fries 1832, De Bary 1836), Hemibasii (Dietel 1928) and Teliosporeae (Bessey 1935), but none of these has the ending (-mycetes) characteristic of a class of fungi, while the term Promycetes suggested by Clements and Shear (1931) is confusing since its literal meaning is different from the intended one of 'promycelial fungi'. I suggest therefore that the term Coniomycetes, used by Fries (1832) for the Ustilaginales, Uredinales and certain Fungi Imperfecti should be revived and used as a general term for the Ustilaginales and Uredinales as a class. The word Coniomycetes, meaning "dust-fungi", is particularly appropriate to these two orders of fungi.

SUMMARY

With the realisation that the primary function of sexual reproduction in living organisms is the recombination of hereditary differences the essential features of this process, formerly regarded as the fusion of male and female organs, are now looked upon as the occurrence of nuclear fusion and meiosis in the life-cycle This change of view has made possible a fuller understanding of some of the phenomena of sex in the fungi. The following points are discussed :

1. The claim that in some Ascomycetes two nuclear fusions and two chromosome reductions occur in the life-cycle is not substantiated. It is based solely on cytological evidence and in a number of instances this has been contradicted by genetical data. Until reliable evidence, such as that provided by genetical analysis, is obtained in its support, the theory is best regarded as not proven.
2. The non-functional character of the gametangia in some Ascomycetes and their absence in many other Ascomycetes and in the Hymenomycetes and Gasteromycetes, is no indication of sexual degeneration. The association of unlike nuclei necessary for recombining hereditary differences, is achieved by vegetative fusion of hyphae, and

cross fertilisation is ensured by the existence of heterothallism. The presence of multiple-strain heterothallism in the Hymenomycetes and Gasteromycetes, formerly regarded as asexual organisms, shows them to have the most efficient type of sexual reproduction of all fungi. A scheme, showing the possible sequence of evolution of these various phenomena, is included.

3. The Ustilaginales and Uredinales were incorporated by Brefeld and others within De Bary's class Basidiomycetes, and this practice has since been widely followed. Reasons are presented for believing that the Ustilaginales and Uredinales (with two-allelomorph heterothallism) form a natural group, which probably diverged at an early stage from the ancestors of the Hymenomycetes and Gasteromycetes the latter two sub-classes (with mutiple-allelomorph heterothallism) forming a second natural group. It is therefore suggested that a return should be made to De Bary's use of the term Basidiomycetes for the Hymenomycetes and Gasteromycetes alone, and to Fries' use of the term Coniomycetes for the Ustilaginales and Uredinales.

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SPORULATION & AVERSION IN EUROTIUM HERBARIORUM (Wigg)

By D. D. Gupta

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INTRODUCTION

This work on *Eurotium herbariorum* was undertaken to repeat the investigations carried out by Fraser and Chambers (1907) at the suggestion of Professor Dame Helen Gwynne-Vaughan who thought that in view of the advances made in mycological technique the fungus should be reinvestigated from a cytological standpoint. As a result of this work almost all the results recorded by Fraser and Chambers (1907) have been confirmed. Physiological work was also carried out which revealed the phenomenon of mutual aversion between colonies of this fungus.

Aspergillus glaucus was first recorded by Link in 1809. He used this term for the green conidial stages and *Eurotium* for the perithecial forms of *Aspergilli* found upon badly dried specimens in phanerogamic herbaria. De Bary (1854) in his classical researches, carried out thorough investigations to study the morphology of *Eurotium Aspergillus glaucus* and *Eurotium repens*. It may be stated that *Eurotium Aspergillus glaucus* is now known as *Aspergillus herbariorum* (Wigg.) de Bary.

Fraser and Chambers (1907) studied the cytology of this fungus and confirmed as well as in some directions, extended De Bary's observations. Thom and Church (1926) made a very detailed survey of the Aspergilli and divided *Aspergillus herbariorum* into two series—major and minor–based on the size of the ascospores. They emphasised the necessity of single conidial cultures for purity of the strains.

Barnes (1926) studied the variations produced in *Eurotium herbariorum* by the action of high temperature and Chona (1932) investigated a member of the group species, *Aspergillus glaucus* from a physiological point of view. The effects of different concentrations of sugar and nitrogen in the medium on the growth of the fungus were observed and reference is also made to the effect of temperature and light. The name of the species has not however been stated. So far as is known, no successful attempts have been made to isolate mono-ascospore or mono-conidial cultures of *E. herbariorum*.

Barnes (1928) made transfers of large numbers of conidia. Regarding the difficulty of isolating single spores of *Eurotium herbariorum*, he writes–" A satisfactory method of working with single spores has not been devised... ..". Further he states " The behaviour of ascospores has not been investigated. The stock strain of *Eurotium* forms conidia freely, so that it is difficult to isolate a perithecium free from conidia. The microscopic examination necessary to ensure this involves a good deal of handling, and so introduces a serious risk of contamination ". He isolated twelve strains from single perithecia but all were contaminated.

EXPERIMENTAL RESULTS

The strain used throughout the work was that isolated by Barnes in 1921 from a growth on the remains of a cigarette. Two tubes of this strain were received from Birkbeck College, London, by the courtesy of Dr. Barnes.

It was grown on prune agar, and besides producing abundant conidia, produced perithecia so abundantly at 30°C that the cultures looked perfectly yellow after 6 to 8 days.

At first the young colonies appear snowy white due to conidial production which later turn slaty grey. A region of young conidiophores is seen just inside the margins of the advancing hyphae. Perithecial development starts in the centre of the colony, but advances very rapidly so that the whole colony soon gets covered with yellow coloured perithecia.

Throughout the work the culturs were grown in Petri dishes, on prune agar containing 20–40% cane sugar. In the early stages of the work 40% cane sugar was used. This caused difficulty in setting and the quantity was reduced to 20–25%.

For the " aversion " trials the following medium was used :

Decoction of 12 prunes made upto ... 500 c. cs.
agar agar ... 25 gms.
cane sugar ... 150 gms.

The medium and Petri dishes were sterilised in the autoclave at 130°C and 25 lbs. pressure for 20 minutes.

CYTOLOGY OF THE GAMETOPHYTE

It has been observed that in some cases normal fertilisation may take place but in most cases the antheridium seems to be functionless because it is found to remain outside even where the wall of the perithecium has formed. Some indications of parthenogenesis and homeogamy have been observed. The ascospores at first contain a single nucleus, which then divides to form eight nuclei.

In one of the instances it was observed that a young perithecium showed a single ascus containing only four ascospores. This reminds one of a similar case cited by Gwynne Vaughan and Barnes (1927) for *Eremascus fertilis*. They say " Pairs of uninucleate cells grow up from the same or different hyphae their nuclei fuse and after three karyokinetic divisions eight spores are formed. Sometimes especially in old cultures, the fertile hyphae may produce asci without fusion; these are usually smaller than the ordinary asci and contain four or fewer spores ". This was regarded as a case of parthenogenetic development of the asci. Since, in the instance described, only four mature ascospores are developed, it seems suggestive that no sexual fusion took place in the sense described by Fraser and Chambers (1907). As evidence, the rudimentary antheridium was found at the base of the perihecium. It seem likely, however, that there may have been a homeogamous fusion in the ascus–two female nuclei fusing to form the nucleus of the ascus–followed by a single meiosis without a third karyokinetic division ; this resulting in the formation of only four spores. Alternatively, it may be suggested that this

is a case of parthenogenesis as found to occur in *Eremascus fertillis* and *Endomyces*. If no fusion of the ascus took place the meiotic phase would be absent, the four ascospores in the ascus may thus have been produced as a result of two mitotic divisions of a single haploid nucleus in the ascus thus omitting the meiotic phase. The genetic constitution of these spores would, therefore, be similar.

PHYSIOLOGY

In order to determine the effect of light and temperature on this species (*Eurotium herbariorum*) the following experiments were conducted :—

Twelve petri dishes were inoculated and divided into four groups of three dishes each. After inoculation, the dishes were kept in the incubator at 33°C for germination : as the young colony appeared the groups were put in the respective conditions described below.

I. Dark room, fluctuating temperature of 15 to 17°C.

Under these conditions the rate of growth was very slow and there was a very little increase in the size of the colonies. Conidia were produced but not abundantly. No perithecia were formed.

II. Light-proof incubator at 20°C.

In this case the rate of growth was fair, and conidia were produced abundantly but only a few perithecia were formed in the centre of the colonies.

III. Light-proof incubator at 33°C.

In this case the rate of growth was most rapid and both conidia and perithecia were most profusely produced.

IV. Laboratory bench in continuous light and temperature of about 20°C (due to electric bulbs)

Under conditions of constant light and fluctuating room temperature (raised to 20°C to 22°C by electric bulbs) the rate of growth was higher than those at 20°C in the incubator. Abundant conidia were formed but no perithecia developed. When these cultures were removed to light proof incubator at 33°C, perithecia, particularly on the outer region of the colonies i. e. on new growth, were produced abundantly, rendering this region perfectly yellow.

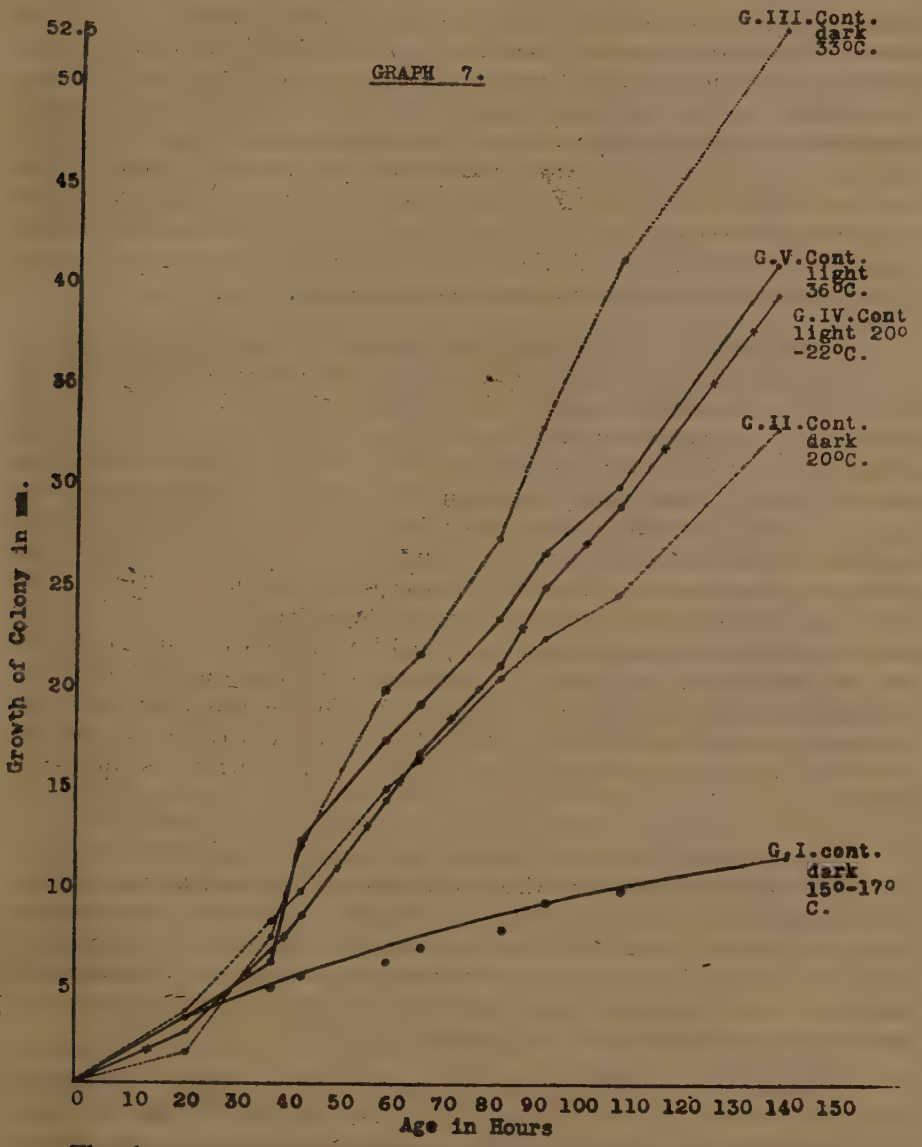
V. Incubator at 28°C and continuous light. For continuous light an electric lamp was put in the incubator which raised the temperature to 36°C.

In the incubator under continuous light, the rate of growth was very rapid in the beginning, but then slowed down. The total growth of the colonies was less than that of the colonies growing in the light-proof incubator at 33°C, but greater than all the rest. The difference, however, was not great. Conidial formation was profuse and towards the end of the growth period, a few scattered perithecia were produced.

Groups II, III and IV were replicated four times and Groups I and V three times.

The diameter of the colonies was used as a measure of growth. Tomkins (1932) considers that for finding approximately the effect of such factors as

temperature on rate of growth, this method is quite adequate. But he suggests that measurements should be made every 12 hours. In this case that procedure was not possible, and growth was, therefore, measured at intervals varying from 4 to 24 hours.



The above results clearly show that vegetative growth is not checked, though the perithecial development is considerably suppressed, by the action of light. In so far as the second effect goes, the results coincide with those obtained by Chona (1932) but do not agree with the former effect, i. e., vegetative growth.

Illumination, therefore, suppresses the formation of perithecia but does not check the vegetative growth as found by Chona (1932) in a related species. At the same time, illumination stimulates conidial production. .

Chona (1932) as a result of some experiments on a member of the group species, *Aspergillus glaucus*, concluded that the one factor which influenced the growth form of the organism, was the concentration of the medium, more particularly as regards sugar. Reference was also made to the effect of intense illumination. He did not study the effect of temperature in conjunction with illumination but only the sporulation of the organism in different media with varying concentrations of cane sugar and nitrogen. He measured growth after a period of 14 days at 25°C, therefore, any variations in growth rate during that period could not be observed. As a result, he found that strong illumination repressed vegetative growth and perithecial formation but stimulated conidial formation; darkness was found to have the reverse effect.

Chona (1932) also found that a temperature of approximately 30°C was the optimum for growth and sporulation of the organism he studied and that the further the temperature was removed from the optimum the slower was its development. A consideration of the graph (Fig. I) makes it evident that the cultures of Group V (continuous illumination at 36°C) grew more slowly than those of Group III (continuous darkness at 33°C) but slightly more rapidly than those of Group IV (continuous illumination at 20° – 22°C). Taking into account the effect of temperature first, it may be suggested that as the cultures of Group III show the maximum rate of growth, the conditions (darkness at 33°C) under which these cultures were grown were very near the optimum. The slightly slower rate of growth of the cultures of Groups IV and V may have been due to the fact that the temperature was below the optimum in the one case and above the optimum in the other. Another factor operating in the two cases was light. Comparing Groups II and IV at 20° – 22°C, light seems to have accelerated the growth rate, whereas in Groups III and V at 33° to 36°C, light has apparently retarded it.

Since, however, it was not possible to keep the temperature absolutely constant in the parallel series growing in darkness and light and keeping in view the apparent sensitiveness of the fungus to changes of temperature, it is considered highly probable that a variation in temperature was responsible for the change in the rate of growth in the groups of cultures under discussion. It is concluded, therefore, from these experiments that light had little, if any, effect upon the rate of growth as measured by the diameter of the colony.

Undoubtedly, the production of conidia and perithecia in *Eurotium herbariorum* entirely agree with the results obtained by Chona (1932), i. e. by strong illumination the conidial formation is stimulated, but perithecial formation is suppressed, and that when the cultures kept under constant illumunation are removed to conditions of darkness at 33°C perithecia appear very readily on the peripheral regions of the colonies, as well as, to a certain extent, in their central

parts. It seems that, though the perithecial initials are formed, they are unable to develop any further. This may be due either to the smothering effect of profuse conidial growth ; or to the depressing effect of light.

Working with *Sphaeropsis malorum* and *Fusarium*, sp., Brown (1923) states– " The general feature of these curves (of the rate of growth as measured in terms of the diameter of the colony) is that in the early stages the rate of growth is small and that it then rises to a maximum which may or may not be maintained. Fungi which keep up this limiting rate of growth are described as being of the staling type " Results of the present experiments, however, agree with those of Tomkins (1932) who considered that staling was an exception and constant linear growth the general rule. In *Eurotium herbariorum* also, constant linear growth is found to occur.

" AVERSION " IN *Eurotium herbariorum*

In the course of these studies, the phenomenon of " mutual aversion " or " antagonism " between colonies of the same fungus was observed. To investigate the phenomenon one of the petri dishes containing several inocula was placed under the low power of the microscope to observe the growing hyphae. It was found that the hyphae were growing very rapidly. Some of these hyphae were diagonally opposite to one another. Several such hyphae in a course of three hours had gone past each other; but one pair behaved differently. At the beginning, the rate of growth of this last named pair, growing in diametrically opposite directions, was very rapid, but it soon slowed down and later it ceased. The petri dish was kept under observation at room temperature on the stage of the microscope for two days, but no further growth was observed in these two hyphae while others had been growing slowly. The dish was then removed to the incubator at 35°C and kept for 24 hours and re-examined but no further growth in these two hyphae was observed. Putting the dish at 35°C increased the growth rate of the other hyphae which had grown still further but these particular ones showed no signs of further development.

" Aversion " has been defined as the inhibition of growth at the adjacent edges of colonies of micro-organisms, espe.ially in a culture of one species. It was impossible to test the occurrence of the phenomenon in *Eurotium herbariorum* further, without mono-conidial cultures and for that purpose a techique had to be devised –Gupta (1935). The difficulty of isolating single spores has been appreciated by mycologists and particularly small conidia such as those of *Eurotium herbariorum* which provide special difficulty.

With the aid of the technique developed–Gupta (1935) twenty-three strains were obtained, each grown from a single conidium. Some of these showed signs of contamination and were, therefore, discarded, leaving 15 pure mono-conidial cultures.

All these mono-conidial cultures produced well developed perithecia readily and abundantly, showing that *Eurotium herbariorum* is hyplo-synoecious or homothallic as regards sex. One such culture $\frac{5}{3}$ 12 is shown in Fig. 1.

CHART I

$\frac{5}{3}2$	5	6	7	8	12	13	14	15	16	17	18	19	20	21
$\frac{5}{3}2$	0	-	?	+	-	0	0	+	+	?	0	0	+	0
5		-	0	+	+	0	+	+	+	+	?	?	0	+
6			-	-	0	+	?	+	?	-	0	0	?	+
7				+	-	0	+	0	0	?	0	+	+	+
8					-	?	-	+	0	?	+	+	0	+
12						0	+	-	0	0	0	+	+	+
13								0						
14									-			0		-
15									+	+				-
16										+	+	0	-	-
17											0	-	+	?
18												0	0	0
19													0	-
20														+
21														

+ = Colonies meeting
- = "Aversion"
? = Doubtful cases showing various degrees of aversion
Blank or 0 = No result or unsuccessful inoculation.

Chart I. - Showing the behaviour of 15 Mono-conidial test strains towards one another

Some of the above combinations which were tried again gave the same results

All cultures were inoculated on the same day, grown on the same concentration of the medium (prune agar), kept under similar conditions and examined on the same day.

The 15 monoconidial cultures were tried in all possible combinations. Each Petri dish was inoculated with two strains placed at a more or less constant distance from each other. The colonies that developed showed varying degrees of the suspected aversion. Those cultures which met freely were marked with a positive (+) sign while those that did not meet with a negative (-) sign.

Some cultures were grown in which the inoculum used at two points was derived from one and the same monoconidial culture such as $\frac{5}{3}$ 5x5 (Fig. 2).

PLATE I

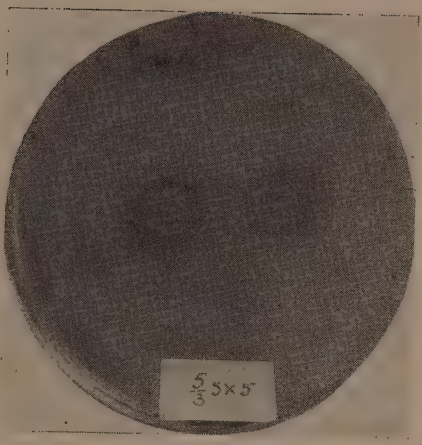


PLATE II

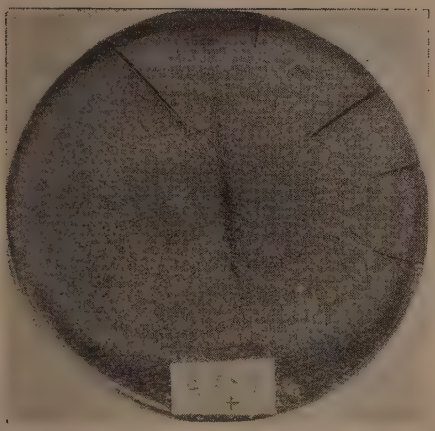


PLATE III

PLATE IV

In some combinations it was found that the mycelia of the resulting colonies intermingled quite freely, while in others there was a varying degree of aversion, that is, in some cases the line of demarcation was not pronounced and certain small groups of mycelia were found to pass to a short distance towards the other colony (Fig. 3). Combination $\frac{5}{3}$ 6 x 17 (Fig. 4) showed a very broad and marked zone of agar, free from any growth of the mycelium, indicating a stronger aversion in this case.

In *Diaporthe perniciosa* Cayley (1923) found that if two pieces of mycelium from one and the same averting monoascospore strain were tested together, the colonies intermingled and showed no aversion. I found this to be precisely the case in *E. herbariorum* also. Culture $\frac{5}{X}$ 5 x 5 (Fig. 3) shows such a combination. In most cases, however, the mycelium seemed to intermingle slightly, (in some cases more freely) where the marginal extremities of the two colonies came in contact with each other.

No definite classes or groups of averting and non-averting strains could be suggested at this stage of the work. Neither any scheme or schemes could be put forward to illustrate th phenomenon, nor its relation to genetics be emphasised as done by Cayley (1923) for *Diaporthe perniciosa.*

As there is no apparent staling effect produced in the medium (growth rate being constant and linear), it is suggested that there are marked indications of aversion in this species. But, as the cultures are monoconidial and, therefore, of a multinucleate origin, there is to be found a greater degree of variability. It, however, cannot be asserted that aversion in *Eurotium herbariorum* is so clearly defined as described by Cayley (1923) for *Diaporthe perniciosa.*

Derx (1926) found true sex heterothallism (simple heplo-heteroecism) in *Penicillium luteum* in which, cultures derived from a single spore did not produce perithecia *E. herbariorum* which is shown to be a homothallic species as regards sex, aversion is probably purely a manifestation of physiologic strains.

Penicillium luteum provided the first example of true sex heterothallism in that genus as described by Derx (1926). *Eurotium herbariorum* provides the first example of the suggested occurrence of the phenomenon of aversion in the genus *Eurotium.*

It is hoped that combinations from isolated ascospore cultures will show more clearly the exact nature of aversion and indicate more precisely whether it is a physiological or a genetical problem. Investigation of the behaviour of monoascospore cultures is in view.

SUMMARY AND CONCLUSIONS

1. The physiology and cytology of *E. herbariorum* have been investigated, which confirm the work of Fraser and Chambers on the morphology of *Aspergillus herbariorum.* Uninucleate conidia also occur.

2. The antheridium in some cases arises at a very early stage of development of the archicarp as a side branch of its stalk. In some examples the

antheridium takes no part in the development of the perithecium and it seems probable that, in some cases, parthenogenetic development of the ascus occurs.

3. Rate of advance of the colonies under all conditions is more or less constant. *Eurotium herbariorum* is of the non-staling type and growth rate, conidial and perithecial production in *Eurotium herbariorum* is greatly affected by temperature.

4. Low temperature even in continuous darkness retards vegetative growth and the production of both kinds of fruiting structures. Temperature of about 30°C and continuous darkness are most favourable for vegetative growth, and the production of both kinds of reproductive structures but continuous illumination has little or no effect on the rate of advance of the colonies.

5. Continuous illumination stimulates conidial production but inhibits perithecial formation irrespective of temperature (i. e. from 20° to 36°C).

6. *E. herbariorum* has proved to be homothallic as regards sex, but gives indications of the phenomenon of aversion. Experiments so far carried out on the phenomenon of aversion show that—

(a) Mycelia derived from one and the same monoconidial culture show no aversion ;

(b) Mycelia derived from different monoconidial cultures give indications of aversion.

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INVESTIGATIONS ON THE EFFECT OF SEED TREATMENT OF FLAX (LINUM USITATISSIMUM L.) IN BENGAL

By T. C. Roy

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A number of pathogenic fungi attack flax plants and cause serious damage. *Fusarium lini* Bolley; *Sclerotinia sclerotiorum* (Lib.) deBary; *Alternaria lini* Dey; *Oidium lini* Skoric; *Macrophomina phaseoli* (Maubl.) Ashby and *Melampsora lini* (Pers.) Lév attack flax in one stage of the host or the other and cause much damage. It has been proved that many of these pathogens are seed-borne. Muskett at the Fourth Commonwealth Mycological Conference in 1948 described methods for examining flax seeds for various fungi including *Colletotrichum linicola*; *Polyspora lini; Bortytis cinerea; Phoma spp.; Fusarium lini*; *Alternaria tenuis* and *Alternaria linicola*. Muskett & Colhoun (1946) found seed from dry regions much freer from seed-borne fungi than seed from wetter parts. Wilson (1946) proved that a relatively high soil temperature favours infection by *Fusarium lini*. As the predisposing factors of prevailing high temperature and humidity exist in the plains of Bengal a fairly high incidence of flax wilt caused by *Fusarium lini* is the most serious menace to successful cultivation of the crop. This led to an investigation to find out the effect of seed treatment with some fungicides on the emergence and incidence of wilt of flax.

Experiments were conducted in consultation with the Fibre Expert on the effect of seed treatment with three different fungicides e. g. (a) Agrosan G ; Nomersan and (c) Copper sulphate, on flax var. J. W. S. The following treatments were given :

(a) Seeds dressed with Agrosan G at the rate of 2 oz. per 56 lbs. of seeds.

(b) Seeds dressed with Nomersan at the rate of 2 oz. per 40 lbs. of seeds.

and (c) Seeds dipped in 2 per cent copper sulphate solution for 10 minutes and then dried quickly in thin layer in the sun.

Seeds were sown in randomised blocks having a plot size of 20′ x 10′. All the plots were uniformly manured with lime and farm yard manure at the rate of 5 mds. respectively to the acre before sowing. The seed rate was uniform in all the plots. Three irrigations were given at an interval of one month.

The experiment was laid out in randomised blocks.

The emergence count was taken after the seedlings came up in the field 4 weeks after sowing.

The results are recorded in the following table :

Table I. *Data of the Emergence count*

Treatments	Block I	II	III	IV	V	VI	Average
Agrosan	11582	7345	15573	10562	9795	11100	10992.8
Nomersan	12828	9916	11914	12446	11722	10386	11535.3
Copper sulphate	10988	8105	9806	9632	11413	10028	9995.3
Control	12355	8387	9959	11465	13265	11912	11223.8

Results of the analysis of variance did not show any significance. It is to be noted that the treatment of the seeds with the different fungicides had no effect on the emergence of the seeds.

Disease counts of the seedlings showing wilt were made by taking 10 spots at random of 1 sq. ft. in each plot. The results are given below :

Table II. *Total No. of seedlings wilted*

Treatments	Block I	II	III	IV	V	VI	Average
Agrosan G	293	287	347	236	327	344	305.67
Nomersan	378	328	396	407	372	287	361.33
Copper sulphate	424	391	412	426	357	357	394 5
Control	617	519	567	579	682	581	590.83

Table III *Analysis of variance (diseased count)*

Block	5	9732.33		
Treatment	3	276940.83	92313.61	46.887**
Error	15	29532.67	1968.84	
	23	316205.83		

** Significant at 1% level

Table IV. *Average No. of diseased plants*

Agrosan	Nomersan	Copper sulphate	Control	S. E.	C. Diff. 5%
305.67	361.33	394.5	590.83	18.11	31.966

The analysis of variance showed that the treatments produced results significant at 1% level. It was found that according to the effectiveness of the treatments they may be graded in the following order :

Agrosan G Nomersan Copper sulphate Control

These findings, however, do not agree with those found by Cass Smith & Harvey (1946) in Australia where they found significant difference in plant numbers at the emergence stage in cases of seed treatment by Ceresan U 564, Nomersan, Agrosan, Ceresan U. T. 1875A and Spergon. Cass Smith & Harvey's findings showed the superiority of Nomersan to Agrosan in controlling wilt. Wilson (1946) working at Aberystwyth on Bison seed however found that Nomersan reduced *Fusarium lini* from 14 to 3 per cent, Agrosan G to 1 per cent and as such was superior to Nomersan. The present findings are in line with those of Wilson and show that seed treatment of flax with Agrosan G gives significant result in reducing the incidence of wilt.

I am indebted to Dr. B. B. Mundkur for helpful suggestions and for a critical reading of the manuscript.

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THE GENUS TROGIA Fr. IN INDIA

By R. W. G. Dennis

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The genus Trogia was founded by Fries (1836) in the following terms :

" Lamellae rigescentes, plicaeformes, acie compaginatae, obtusae et longitudinaliter canaliculatae. Habitus Schizophylli, sed lamellulae apice haud discretae et revoluae, sed potius involutae et acie conjunctae. Uc tropicum (typo Canth. aploruti Montagn.) mihi tantum cognitum genus ".

Cantharellus aplorutis Mont., thus chosen by Fries as the type of his new genus, was described from a collection made by M. Belanger on dead and fallen branches at Trichinopoly, March 1826. Montagne's description runs as follows :— " Espéce haute d'un pouce à un pouce et demi, y compris le stipe, qui a tout au plus 3 lignes. Son chapeau trés mince, en entonnoir. à bord sinueux, acquiert jusqu' à 2 pouces de diamétre. Les plis droits, inégaux, espacés, la plupart simples, car on n' en voit que deux ou trois qui se bifurquent prés du bord, se dessinent un peu en relief à la face supériure comme a l' inférieure, mais d'une maniére beaucoup moins prononcée cependant. et sont morqués d'un sillon qui les parcourt dans toute leur longueur, disposition qui a persisté par le sejour du champignon dans l'eau, et qui offre un passage naturel entre les genres Cantharellus et Schizophyllum Fr. La couleur de cette espéce est d'un jaune abricot à l'etat de vie, d'un bai roux à l'état de dessication ".

As the original publication is somewhat rare I reproduce Montagne's figure of *C. aplorutis* as Fig. I beside a sketch of one of the two dried sporophores in the type collection at Paris. The furrowed gill which led to Montagne's suggestion of an affinity with *Schizophyllum* is the feature on which *Trogia* was founded but examination of the type shows this to have been a misinterpretation. The gills are very narrow, little more than folds, but their edge is evenly rounded. Microtome sections show that the entire trama is built up of very slender hyphae about 1.5 μ wide, embedded in a gelatinous matrix. At the pileus surface these hyphae are closely packed, elsewhere they are loosely woven. There is a thick subhymenial layer, apparently not gelatinised. The basidia are not well preserved and are possibly immature as no sterigmata can be found but they seem too small to be heterobasidia of the Auriculariaceous type. The gelatinous trama emerges in a narrow belt down the gill edge which appears as a whitish line when the soaked up gill is viewed from below by transmitted light and this no doubt gave rise to the conception of a bifurcation. No spores were seen and there are no pleurocystidia.

In 1838 Fries renamed the type species *Trogia montagnei* Fr., a step which is inadmissable under the present rules of nomenclature, and added to the genus two more Indian fungi viz. :—

T. berlangeri, pileo subsessili coriaceo reniformi ferruginèo–fusco, basi verrucoso, lamellis confertis subfurcatis fuscis priunosis. Montagn. in Berl. Voy. p. 145. t. 14 f. 4. Ad truncos in Gatibus occident. Indiae. .....

T. Konigii, pileo sessili membranaceo–lento obovato laevi glabro fuscescente, lamellis plicaeformibus subconfertis inaequalibus–furcatisque pallidis nudis. In India orientali. Konig. .....

T. berlangeri, as shown by the figure quoted, is a mispelt recombination of *Agaricus belangeri* Mont., published in the same work as *C. aplorutis*. Bélanger's book is undated. In the catalogue of the library of the British Museum, Natural History, the date is given as 1846 but this must be an error as Plate 14 was cited by Fries (1838) and Plate I by Endlicher Gen. p. 625, also in 1838.

I have been unable to trace material of *T. konigii*. Prof Nannfeldt kindly informs me there is none in Fries' herbarium at Uppsala, but there is at Kew an authentic specimen of *T. belangeri* sent by Montagne to Berkeley. It consists of a single slightly convex pileus about 11 mm. diameter and liver brown (Ridgeway) in the dry state, the upper surface is areolately cracked, the gills narrow, crowded, concolorous with an obtuse, minutely pruinose edge. As in *C. aplorutis* there is no real furrow along the gill edge, merely a darker central line fringed on each side by a paler pruinose margin. Microtome sections show that there is a pellicle about 35 μ thick on the pileus surface composed of slender rather closely woven hyphae 2 μ wide, overlying a trama of similar slender hyphae with very thick, hyaline, gelatinised walls. In the pileus the individual gelatinous sheath of each hypha is easily distinguishable but in the gills the slender hyphae appear to lie in a structureless matrix. The subhymenium is about 10 μ thick, of slender, closely woven, non–gelatinised hyphae, basidia are cylindric-clavate, about 12x2.5, μ probably 4–spored, the spores elliptical, hyaline, 4 x 3 μ. The gill edge proves when soaked up to be formed of loose slender hyphae embedded in colourless mucilage. It is no doubt the shrinking of this mucilaginous mass between the parallel hymenia which gives the slightly canaliculate appearance to the margin when dried.

The spore reaction cannot be determined in the type of *Ag. belangeri* but a recent West Indian collection on sticks, High Peak, Jamaica, which differs in ascertainable characters only in its tomentose less warted surface, has yielded a white spore print, strongly blue–amyloid, with spores 4–5 x 2.5 3 u.

Two species anatomically closely akin to *Ag. belangeri* are *Panus bicolor* Mont. (1854) and *P. sprucei* Berk. (1856), both from tropical South America. All these fungi have similar tramas and gill edges but differ from *C aplorutis* in their much better developed, more closely spaced gills. Whether they are congeneric with it can hardly be decided until it has been recollected and its basidia and spores are known. Certainly none of them bears any resemblance either in anatomy or in gross morphology to *Schizophyllum*. *Ag. belangeri* and its allies do, however, fall readily into *Pleurotus* in the wide interpretation adopted by Pilat (1935) though the combination of amyloid spores and a completely gelatinised trama and gill edge does not fit any of the segregates from that genus

proposed by modern authors. From *Panus* Fr. in the sense of *P. stypticus* (Bill ex Fr.) the type species selected by most authors and indirectly indicated by Fries himself, they differ in their gelatinous flesh. From *Scytinocus* Karsten they are distinguished by their small amyloid spores. Actually their closest affinity appears to be with *Dictyopanus* Pat. but the poroid hymenophore of *D. pusillus* (Lev.) Sing. is very different in gross morphology from the normal gills of *Ag. belangeri*. I propose, therefore, to transfer this small group of species to Pleurotus (Fr.) Kumm, sensu lato as *P. belangeri* (Mont.) comb. nov. *P. bicolor* (Mont.) comb. nov. and *P. sprucei* (Berk.) comb. nov.. Because of their anatomical similarity I propose also to treat the Jamaican collection as a variety of *Ag. belangeri* under the name *Pleurotus belangeri* var *occidentalis* var. nov., a typo differt pileo tomentoso. Ad ramulos dejectos High Peak, Blue Mountains, Jamaica, 22 December 1949, Dennis J 49. Typus.

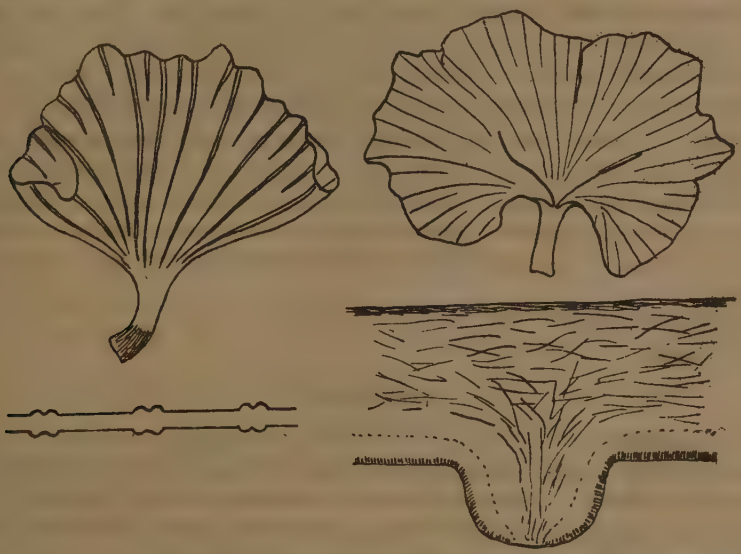

FIG. 1. *Cantharellus aplarutis* Mont. **Left:** Montagnes Figure and Section of the sporophore. **Right:** Sketch of the type at Paris and diagrammatic section of pileus and gill X 45.

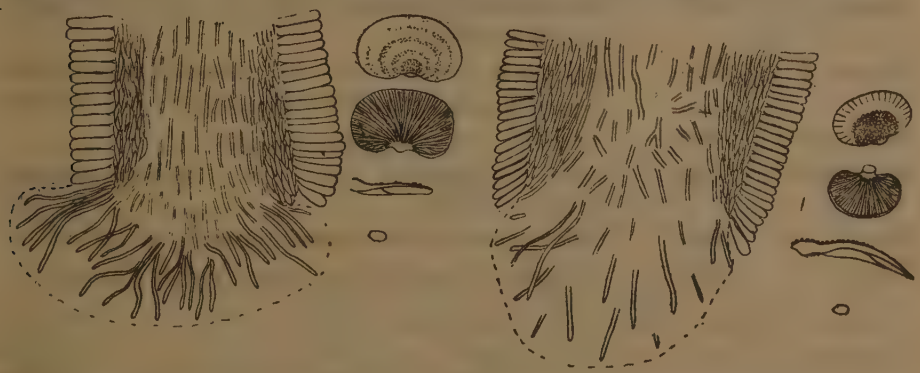

FIG. 2. **Left:**—*Pleurotus belangeri* and *occidenkalis*, section of gill edge and spore X660, upper and lower view of sporophore and section XI. **Right:** Type of *Ag. belangeri* mont, gill section and spore From Fregment at kew, X660, upper and lower surtace of sporophore and section after montague XI.

Unfortunately the subsequent history of *Trogia* was still further complicated by Fries' transfer to it in 1863 of *Cantharellus crispus* Pers ex Fr. a well known North European fungus with sessile pilei on dead branches. This species has a completely non-gelatinous trama, of loosely woven hyaline hyphae 5 μ wide, with thick walls near the pileus surface, thin walls elsewhere, very small basidia and minute cylindrical or slightly curved spores about 2 x 1 μ. This is evidently congeneric neither with *C. aplorutis* nor with *Ag. belangeri* and is today usually referred to the genus *Plicatura* Peck. founded on *P. alni* Peck, Patouillard [1900] has transferred to *Trogia Cantharellus buccinalis* Mont., *Panus cantharelloides* Mont , *Cantharellus partitus* Berk, and *Xerotus griseus* Berk. By courtesy of the Director of the Museum National d'Histoire Naturelle, Paris, I have been able to examine the type collections of the first two of these. *C. buccinalis* has a trama like *Ag. belangeri*, with thick gelatinous walls to the hyphae. Its gills have been much flattened and their margin could not be recovered. They are certainly narrow but much more closely spaced than the fold-like gills of *C. aplorutis*. *P. cantharelloides* has narrow crowded gills but they are thin, have no sterile gelatinous margin and the tramal hyphae are apparently non-gelatinised.

Both *C. aplorutis* and *Ag. belangeri* should be easily recognisable from the descriptions and figures reproduced and it is to be hoped Indian mycologists will be able to recollect them in or near their type localities and to obtain spore prints from the fresh material. Only then can we be sure of their true affinities and have a clear understanding of that misconceived and abused genus *Trogia* Fr.

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STUDIES IN SOME SPECIES OF XANTHOMONAS

By M. K. Patel, G. W. Dhande & Y. S. Kulkarni

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INTRODUCTION

Amongst the phytopathogenic bacteria that have so far been studied in India, a majority belongs to the genus *Xanthomonas*. They cause leaf-spots on various species of plants and are parenchymatous forming a separate group unlike those belonging to the genera *Corynebacterium*, *Erwinia*, *Pseudomonas* and *Bacterium*. The individual species of *Xanthomonas* although possessing the morphological and cultural characters common to the genus, have been studied in considerable detail by workers in this laboratory. But a study of all of them together and under identical conditions has not been made and it was considered desirable that such a study would yield interesting data. An attempt has, therefore, been made and to classify them, if possible, in one or more groups on the basis of their morphological, physiological and serological characters and host specificity.

SOURCE OF CULTURES

The cultures used in the present work were maintained on neutral potato dextrose agar and often tested for purity and pathogenicity.

MORPHOLOGICAL AND CULTURAL REACTIONS OF XANTHOMONAS SPP.

Morphology and Staining Reactions

All the organisms were true to type being rod-shaped, single or in pairs, non-spore formers and not acid-fast. The motility of the isolates was determined by hanging drop method in a drop of peptone broth. They were also stained by the flagella staining method developed by Patel, Kulkarni and Gaekwad (1950). All were found to be motile, possessing 1 or 2 polar flagella. Both young and old cultures when stained by Kopeloff's and Beerman's modified gram stain method were found to be gram negative and capsulated when stained by Hiss' method.

CULTURAL CHARACTERS

The cultural characters of the organisms were studied on various media prepared according to the procedure prescribed in the Manual of Methods (1946). The cultures were incubated at room temperatures i. e. 27°-30°C. unless otherwise mentioned.

Nutrient agar plates.—All the cultures grew rather poorly except *X. badrii* and *X. alfalfae* which showed better growth. The colony diameter ranged from 6 to 8 mm. with a shining halo around. Colour of the colonies was predominantly yellow and were indistinguishable.

Nutrient broth:—The growth on this medium was slightly better since all the cultures grew fairly well without forming pellicles except *X. badrii* and *X. malvaceurum*. Colour of the medium remained unchanged and odour was absent.

Nutrient dextrose agar plates:—This medium was found to be quite suitable for growth of all the organisms. Colonies were circular with some shade of yellow and entire margin. Colour of the medium did not change even on ageing.

Potato dextrose agar plates:—All species grew copiously on this medium as compared to others, with yellow pigments more intense. The growth was smooth, raised, with entire margins and shining.

Potato cylinders:—*X. desmodii*, *X. desmodii-gangeticii* and *X. malvacearum* grew more copiously than others. The cylinders in most of the cases were discoloured to light gray.

It will be seen that it is not possible to differentiate species of *Xanthomonas* under study since their growth on various media is fairly similar.

Gelatine:—Stab cultures on nutrient gelatine and plates with Frazier's gelatine were inoculated, incubated and observations made after 96 hours. All hydrolysed gelatine by producing zones around the colonies. The results are in confirmity with those reported by Dowson (1949).

Starch :—Plates poured with nutrient agar containing one per cent potato starch, inoculated and incubated for 4 days were flooded with a weak solution of iodine. A c ear zone around the colony was present in each case, the zone varying from 1.5 to 3 mm. indicating starch digestion. It may be noted here that a majority of *Xanthomonas* spp. attacks starch (Dowson, 1949).

Hydrogen sulphide:—Tubes containing the medium prepared according to Levine (1933) were inoculated and incubated with strips of filter paper impregnated with lead acetate between the cotton plug and the lip. Observations made after 4 days showed that all the cultures produced hydrogen sulphide, as indicated by darkening of the filter papers.

Indole:—None of the *Xanthomonas* spp. could produce indole in nutrient broth containing 0.01 per cent tryptophane when tested with Gore's reagents.

Ammonia:—All the cultures reduced nitrates to ammonia.

Nitrates:—The ability to produce nitrites from nitrates is normally restricted to species of *Bacterium* as a whole and half of *Pseudomonas* species but rare in spp. of *Xanthomonas*. The test for nitrites was made after 5 days. None of the *Xanthomonas* spp. could reduce nitrates to nitrite. It may be that bacteria producing nitrites have a wider range of host plants and since the species under study are specific in their pathogenicity as will be shown later in this paper, they are not able to produce nitrites.

Litmus milk :—Duplicate tubes of litmus milk were inoculated and incubated for 3, 5, 10 and 30 days after which observations were recorded. The results show that a definite acid reaction was produced by all the cultures. *X. campestris*, *X. desmodii* and *X desmodii-gangaticii* reduced litmus completely in 8 days, whereas *X. vignicola* and *X. vesicatoria* were rather slow in their action.

The *Voges-Proskauer* and *methyl-red tests* :—All the cultures were negative to both these tests. It may be pointed out that a large majority of species of bacteria as a group produce positive V. P. test, while others seem to be negative. This finding seems to agree with that found in nitrate reduction.

Lipolytic medium :—In order to test the ability of the organisms to utilise fats or oils to form acids to be detected by a colour reaction, the medium according to Starr and Burkholder (1943) was prepared. The cultures did not produce typical bluish halo indicating that lipase was absent. The results are somewhat contradictory to those of Dowson (1949) who states that the genus *Xanthomonas* possesses marked lipolytic activity.

Citrate utilisation:—All the cultures grew poorly on this medium indicating that none could utilise citrate as a sole source of carbon.

Asparagin medium :—A synthetic medium as devised by Starr and Weiss (1945) was used. The results recorded in table VI show that *Xanthomonas* spp. do not show any appreciable growth in the medium, indicating that both the carbon and the nitrogen are not obtained from asparagin. It may be, however, pointed out that *Xanthomonas* can utilise asparagin as a source of nitrogen when with dextrose. These results agree closely with those of Starr (1946) and Bhide (1949).

Loeffler's blood serum :—Probably Smith (1920) was the first to show the utility of this medium in distinguishing different species of phytopathogenic bacteria. The tubes containing the medium were inoculated and incubated for 10 days but the readings were taken every alternate day. The results recorded in table I clearly show that all the organisms liquefy blood serum to some extent. Liquefaction starts rather slowly for all the organisms but at the end of 10 days, *X. alfalfae* is the only culture with poor liquefaction. This can be utilized in separating *X. alfalfae* from the seven other species of *Xanthomonas*.

Koser's uric acid :—The organisms showed no appreciable growth in this medium showing their inability to break up uric acid for obtaining their nitrogen requirements.

Sodium chloride: —Smith (1920) and later workers on phytopathogenic bacteria and Burkholder and Starr (1948) have shown the usefulness of sodium chloride in differentiating generic characters. The last named authors (1948) have stated that species of *Xanthomonas* as a group tolerate sodium chloride up to 3 per cent whereas *Pseudomonas* does not tolerate concentrations higher than 2 per cent. Our results show that they could grow well upto 3 per cent concentration.

Table 1 *Growth of Xanthomonas spp. on solidified blood serum*

Liquefaction after days–	Organisms							
	Xanthomonas campestris	*Xanthomonas desmodii*	*Xanthomonas desmodii–gangeticii*	*Xanthomonas vignicola*	*Xanthomonas vesicatoria*	*Xanthomonas alfalfae*	*Xanthomonas badrii*	*Xanthomonas malvacearum*
2 days	+	++	++	+++	+	+	++	++
4 days	+	++	++	+++	+	+	++	++
6 days	++	++	+++	+++	++	+	+++	++
8 days	++	+++	+++	++++	+++	+	++++	++++
10 days	++++	++++	++++	++++	++++	+	++++	++++

Note :— + — Slight liquefaction;
++ — Intermediate liquefaction;
+++ } — Complete liquefaction.
++++ }

Synergism:—The results on synergism clearly showed that 2 or more cultures when grown together do not produce gas within 6 days.

UTILIZATION OF CARBON COMPOUNDS

The literature on the utilisation of carbon compounds by phytopathogenic bacteria is rather confusing since different workers have employed different techniques. For instance, the composition of the basal medium employed in such studies is not mentioned, leading to conflicting results with the same organism as has been shown by Smith (1920), Burkholder (1932) and Lewis (1930). Ammonia produced from peptone by certain organisms neutralises acid produced from a carbohydrate under test, thus giving erroneous results. Burkholder (1932) used a synthetic medium to study carbon utilisation by *X. campestris* and related organisms, with an idea to separate them from others ordinarily not differentiated when peptone was incorporated in the medium. Lewis (1930) thus got conflicting results with *X. malvacearum*. In the present study, therefore, it was decided to use a synthetic medium for carbohydrates recommended in Manual of Methods (1946). The results though taken every alternate day, only the final readings are given in table II.

It will be seen from the table that all the cultures ferment with the production of acid, arabinose, dextrose, maltose, lactose, sucrose, glycerol, dextrin and mannitol while none grew in salicin and only *X. compestris*, *X desmodii*, *X. desmodii–gangeticii* and *X. vignicola* fermented dulcitol. On the whole, the growth of the organisms in arabinose was slight. As far as the organic acids are concerned,

all grew in citric acid but not in oxalic, while *X. alfalfae* grew in acetic and tartaric acid, and *X. vignicola* in acetic acid only. These results are in perfect confirmity with those of *Xanthomonas* spp. reported by Dowson (1949) wherein he has also found the unavailability of salicin and utilisation of lactose.

Table 2— *Fermentation of Carbon Compounds*

	Organisms							
	Xanthomonas campestris	*Xanthomonas desmodii*	*Xanthomonas desmodii-gangeticii*	*Xanthomonas vignicola*	*Xanthomonas vesicatoria*	*Xanthomonas alfalfae*	*Xanthomonas badrii*	*Xanthomonas malvacearum*
Arabinose	+ Sl. gr.	+ Sl. gr.	+ Sl. gr.	+ Sl. gr.	+	+	+ Sl. gr.	+
Dextrose, Maltose, Lactose, Sucrose, Dextrin	+	+	+	+	+	+	+	+
Salicin	–	–	–	–	–	–	–	–
Glycerol	+	+	+	+ Sl. gr.	+	+	+	+ Sl. gr.
Dulcitol	+	+	+	+ Sl. gr.	–	–	–	–
Mannitol	+	+	+	+	+	–	+	+ Sl. gr.
Organic acids: Acetic	–	–	–	+	–	+	–	–
Citric	+	+	+	+	+	+	+	+
Tartaric	–	–	–	–	–	+	–	–
Oxalic	–	–	–	–	–	–	–	–

Note : + = Acid; – = No growth; + Sl. gr. = Slight growth

UTILISATION OF ORGANIC NITROGEN

In previous pages, it has been pointed out that *Xanthomonas* spp. under study make very slight growth in a synthetic medium containing asparagin and none at all in Koser's uric acid medium. It appears from this that the organisms require complex organic nitrogen. In order to study growth of cultures by providing various amino acids as sole source of nitrogen, synthetic medium as used by Bhide (1949) was prepared with and without dextrose. Mushin (1938) who studied the food requirements of certain plant pathogenic becteria found that there

are some compounds containing organic nitrogen which serve both as a source of carbon and nitrogen. Good amount of work as to the nitrogen requirements of wilt bacteria has been reported by Stapp (1930) and Bhide (1949), but practically no work has been so far reported with reference to parenchymatous bacteria.

To the above basal medium various organic nitrogen compounds mostly amino acids, were added in the proportion of 0.1 per cent except tyrosine and tryptophane which were added at the rate of 0.01 per cent.

It will be seen from the results given in table VI that only glutamic acid supported growth of all the organisms even without dextrose, indicating that this acid supplies both carbon and nitrogen. Other amino acids do not allow growth of any cultures in the absence of dextrose, indicating that the organisms can obtain nitrogen but not carbon. Bhide (1949) while working with wilt bacteria of different generic ranks found that as a group, *Corynabacterium* did not utilize glutamic acid for its carbon and nitrogen. On the other hand, the two *Xanthomonas* spp., one *Preudomonas* and one *Bacterium* used it as the only source of carbon and nitrogen. He also found that most of the cultures did not grow in amino acids without dextrose. These findings of his are in confirmity with those reported in this paper.

BACTERICIDAL ACTION OF CRYSTAL VIOLET

The fact that crystal violet inhibits growth of gram negative bacteria has been taken advantage of by several workers in isolation and classification of micro-organisms. Thus, Patel (1926) used it in a selective medium in isolating *Agrobacterium tumefaciens* and Coons and Strong (1931) used it for differentiating different species and strains of *Fusaria.*

Crystal violet (1 : 100) was added in varying quantities to the synthetic nitrate medium so as to obtain final dilution varying from 1 : 1,250 to 1 : 50,000. The plates were poured with the agar medium, inoculated with the organisms and incubated for 8 days. The results are given in table III.

It will be seen from the table that *X. campestris, X. desmodii* and *X. desmodii–gangeticii* form a group in that they grow in this dye at a dilution of 1 : 2,000. On the other hand, *X. vignicola* and *X. cassiae* are inhibited even at 1 : 50,000 concentration. *X. vesicatoria* and *X. alfalfae* probably form a group unique by themselves in that they grow in a concentration of 1 : 1,250 of the dye. *X. malvacearum* forming a fourth group grows at a concentration 1 : 25,000.

Thus this dye serves as an additional aid besides blood serum and carbon utilisation tests in differentiating species of *Xanthomonas.*

Table III—*Bacteriostatic action of crystal violet on Xanthomonas spp.*

Dilutions	Organisms				
	Xanthomonas campestris / *Xanthomonas desmodii* / *Xanthomonas desmodii gangeticii*	*Xanthomonas vignicola* / *Xanthomonas cassiae*	*Xanthomonas vesicatoria* / *Xanthomonas alfalfae*	*Xanthomonas malvacearum*
1 : 50,000	+	–	+	+
1 : 25,000	+	–	+	+
1 : 16,666	+	–	+	–
1 : 12,222	+	–	+	–
1 : 10 000	+	–	+	–
1 : 8,333	+	–	+	–
1 : 7,143	+	–	+	–
1 : 6,250	+	–	+	–
1 : 5,555	+	–	+	–
1 : 5,000	+	–	+	–
1 : 4,545	+	–	+	–
1 : 3,333	+	–	+	–
1 : 2,500	+	–	+	–
1 : 2,272	+	–	+	–
1 : 2,178	+	–	+	–
1 : 2,083	+	–	+	–
1 : 2,000	+	–	+	–
1 : 1,666	–	–	+	–
1 : 1,428	–	–	+	–
1 : 1,250	–	–	+	–

Note : + = Growth.

TOLERANCE TO SODIUM TAUROCHOLATE

The use of sodium taurocholate in the presumptive test in sanitary bacteriology has been made since a very long time. Probably MacConkey (1900) was the first to advocate the use of this chemical to inhibit several contaminants except the colon typhoid ones. Patel (1929) was the first to take advantage of this chemical in isolating particular plant pathogen. Later, Ivanoff (1933) used this chemical in isolating *Xanthomonas stewartii* from soil and other contaminated material. The readings on Patel's medium show that all the organisms make good growth which can be taken advantage of in isolating these plant pathogens from soil or other contaminated material and also in studying their longevity in soil.

AGGLUTINATION STUDIES

Since the morphological and the cultural tests did not show any clear cut differences amongst different species of *Xanthomonas*, it was thought worthwhile to try serological agglutination tests. The procedure was exactly the same as was followed by Link and Link (1928) and Patel (1929). Each agglutination test was repeated twice and when necessary thrice also to confirm the results which are iven in table IV.

It will be seen from the table that the antiserum of *X. desmodii* agglutinates its homologous antigen upto 1 : 448 besides agglutinating *X. cassiae* and *X. alfalfae* at 1 : 56 and 1 : 112 respectively. Other cultures show no relationship.

Antiserum of *X. cassiae* on the other hand gives a titre of 1 : 1792 for its homologous antigen as against 1 : 28, 1 : 896, 1 : 56 and 1 : 448 titres for *X. campestris*, *X. desmodii*, *X. desmodii-gangeticii* and *X. alfalfae* respectively. Others show no relationship.

Antiserum of *X. alfalfae* gives a titre of 1 : 896 for its homologous antigen while it agglutinates *X. desmodii*, *X. desmodii-gangeticii*, *X cassiae*, *X. vesicatoria* and *X. malvacearum* at titres 1 : 112, 1 : 56, 1 : 224, 1 : 14 und 1 : 56 respectively.

Antiserum of *X. campestris* agglutinates its homologous antigen upto 1:1792 and heterologous antigens of *X. desmodii*, *X. desmodii-gangeticii*, *X. cassiae*, *X. alfalfae* and *X. malvacearum* at titres 1 : 1792, 1 : 28. 1 : 56, 1 : 112 and 1 : 112. respectively.

Table IV — *Unilateral agglutination in Xanthomonas spp.*

Antisera for	Organisms							
	Xanthomonas campestris (Kn)	*Xanthomonas desmodii* (Ps)	*Xanthomonas desmodii gangeticii* (Ba)	*Xanthomonas vignicola* (Ch)	*Xanthomonas cassiae* (Cass)	*Xanthomonas vesicatoria* (Chill)	*Xanthomonas alfalfae* (Alfa)	*Xanthomonas malvacearum* (Cot)
Kn	++++	++++	++	-	+++	-	++++	++++
	++++	++++						
Ps	-	++++	-	-	+++	-	++++	-
		++						
Ba	++++	++++	++++	++++	++++	++++	++++	++++
	+		+	+		+	++	+
Ch	++++	++++	++++	++++	++++	++	++++	++++
	+++	+++	++	++	++		++++	+
Cass	++	++++	+++		++++		++++	-
		+++			++++		++	
Chill	++++	++++	++++	++++	++++	++++	++++	+++
	+	++	+			++	++	
Alfa	-	++++	+++	-	++++	+	++++	+++
					+		+++	
Cot	+	++++	++++	+	+++	+	++++	++++
		+					+	++

In this table : ++++ / ++++ = agglutination at 1 : 1792;

++++ / +++ = agglutination at 1 : 896;

++++ / ++ = agglutination at 1 : 448;

++++ = agglutination at 224; ++++ = agglutination at 1 : 112
+

+++ = agglutination at 1 : 56; ++ = agglutination at 28;
+ = agglutination at 1 : 14.

X. vignicola and *X. vesicatoria* remain almost unaffected by the antisera of *X. desmodii*, *X cassiae*, *X. alfalfae* and *X. campestris*. It may be noted that the antigens of these organisms behave differently with antiserum from *X. desmodii–gangeticii*.

Antiserum of *X. desmodii-gangeticii* like the other two antisera agglutinates all antigens at 1 : 224 titre. *X. vignicola* antiserum likewise agglutinates all the antigens at about 1 : 448 to 1 : 896 except *X. vesicatoria* which is agglutinated only at 1 : 28.

X. vesicatoria gives a low titre somewhat. The antigens of *X. desmodii*, *X. vesicatoria* and *X. alfalfae* give a titre of 1 : 448 whereas *X. campestris* and *X. desmodii–gangeticii* give a titre of 1 : 224, *X. vignicola* and *X. cassiae* agglutinate at 1 : 112 whereas *X. malvacearum* agglutinates at 1 : 56.

Antiserum of *X. malvacearum* gives a titre of 1 : 448 for its homologous antigen whereas a titre of 1 : 14 is obtained for *X. campestris*, X. *vignicola* and *X. vesicatoria* antigens. The antigens of *X. alfalfae* and *X. desmodii* agglutinate at 1 : 224 titre whereas those of *X. desmodii–gangeticii* and X. *cassiae* agglutinate at 1 : 112 and 1 : 56 respectively.

It will be clear from table IV that the antisera prepared from *X. desmodii*, *X. vignicola*, *X. vesicatoria* and *X. malvacearum* are so polyvalent that they agglutinate not only their homologous antigens but also the heterologous antigens more or less. The antisera prepared from X. *desmodii*, X. *campestris*, *X. cassiae* and *X. alfalfae* each form a separate group. Thus, these eight organisms in respect of their antisera and antigen relationships can be placed in 5 groups as stated above. These findings are in close agreement with those of Elrod and Braun (1947) who have shown that *X. vesicatoria* and *X. malvacearum*, the two organisms common with us form a group.

Comparing the behaviour of these organisms antigen-wise, it will be seen that they can also be grouped in 5 categories viz.

(*1*) *X. desmodii*, *X. cassiae* and *X. alfalfae*

(*2*) X. *campestris*

(*3*) *X. vignicola* and X. *vesicatoria*

(*4*) *X. desmodii–gangeticii* and

(*5*) X. *malvacearum*

There, thus, exist closer relationships between *X. vignicola* and *X vesicatoria*, since they behave in a similar manner except in respect of antiserum of *X. vignicola*.

HOST RANGE

The natural hosts of the organisms under reference mostly belong to the families Malvaceae, Papilionaceae, Solanaceae, Cruciferae, Compositae and Convolvulaceae. Plants for inoculation purposes were grown in earthen pots, each pot holding 4 to 5 plants. Inoculations were made by rubbing the organisms and then spraying with water or spraying the suspension directly on wounded or unwounded plants when about 10 to 15 days old. Before inoculation, plants were kept under moist chambers for 12 hours, then inoculated and kept under a bell jar for another 24 hours before removing them to the glasshouse benches. Checks were treated in the same way except that the organisms were not involved. Observations as to the infection were made every third or fourth day for over a month when the plants were usually discarded. In all the cases 3 to 4 trials were made. The results represent the final reading and are given in table V.

It will be seen from the table that the organisms are specific in their infectivity in that they generally infect the plants from which they were originally isolated although plants belonging to unrelated families were also subjected to infection. In this respect the genus as reported by Dowson (1949) is unique. These results do not support the views of Elrod and Braun (1947) who apparently feel that phytopathologists create new species on the basis of hosts from which the pathogen is isolated. The large number of species in this or the other genera of phytopathogenic bacteria is not due to non-pathogenicity on members of other families of plants but to their restricted host range as can be seen from the data presented in table V. It would have been better if they had tried other plants and found something positive before generalising. From the table, it will be seen that *Pisum sativum* is a common host of *X. cassiae*, *X. alfalfae* and X. *badrii*. X. *alfalfae* in addition, infects *Trigonella foenum-graecum* and *Melilotus indica*· X. *vignicola* besides its own host, infects *Phaseolus vulgaris*. Besides the host plants to which the pathogens are specific, species of other genera including those of the family Gramineae have been subjected to infection. However, the bacteria pathogens have maintained their host specificity. When one considers that the pathogens isolated from different members of Papilionaceae are specific to their own hosts, one is at a loss to agree with Elrod and Braun. Inoculation of 30–40 plants belonging to the same and other widely different genera does certainly not constitute a narrow host range.

Table V—*Results of the cross inoculation trials*

Host	Organisms								
	Xanthomonas campestris	*Xanthomonas desmodii*	*Xanthomonas desmodii-gangeticii*	*Xanthomonas vignicola*	*Xanthomonas cassiae*	*Xanthomonas vesicatoria*	*Xanthomonas alfalfae*	*Xanthomonas badrii*	*Xanthomonas malvacearum*
Brassica oleracea caulo-rapa	+	−	−	−	−	−	−	−	−
Desmodium diffusum	−	+	−	−	−	−	−	−	−
Desmodium gangeticum	−	−	+	−	−	−	−	−	−
Vigna catjang	−	−	−	+	−	−	−	−	−
Phaseolus vulgaris	−	−	−	+	−	−	−	−	−
Capsicum annum	−	−	−	−	−	+	−	−	−
Lycopersicum esculentum	−	−	−	−	−	+	−	−	−
Cassia tora	−	−	−	−	+	−	−	−	−
Pisum sativum	−	−	−	−	+	−	+	+	−
Xanthium strumarium	−	−	−	−	−	−	−	+	−
Medicago sativa	−	−	−	−	−	−	+	−	−
Trigonella foenum-graecum	−	−	−	−	−	−	+	−	−
Melilotus indica	−	−	−	−	−	−	+	−	−
Gossypium herbaceum	−	−	−	−	−	−			+
Dolichos lablab									
Dolichos biflorus									
Triticum vulgare									
Oryza sativa	−	−	−	−	−	−	−	−	
Zea mays									
Andropogon sorghum									

Note : + = Infection.

DISCUSSION

An attempt is made in tables V, VI and VII to record pertinent information on growth reactions, cultural characters and host range of *Xanthomonas* causing leaf-spots. It will be seen from the tables that the pathogens are very much

alike in their cultural, morphological, and physiological characters. All of them are gram negative, motile, liquefy gelatine and reduce litmus milk, not produce indol and nitrites, hydrolyse starch, produce hydrogen sulphide and form acid from dextrose, sucrose, lactose and glycerol when grown in synthetic carbohydrate medium; they are unable to utilize sodium citrate but can grow in citric acid. They show differential growth in crystal violet; X. *campestris*, X. *desmodii* and X. *desmodii-gangeticii* could grow at a concentration of 1 : 2,000 of the dye while X. *vignicola* and X. *cassiae* were inhibited even at 1 : 50,000; X. *alfalfae* and X. *vesicatoria* could grow well even at 1 : 1,250 and X. *malvacearum* formed a group in itself since it could grow at 1 : 25,000. It may be noted that these organisms are able to utilize organic nitrogen, mainly amino acids only in the presence of carbon. They utilize glutamic acid as a sole source of nitrogen and carbon.

The results presented re are corroborated by those of Dowson (1949) and Bhide (1949). The latter while studying the stomatal invasion of X. *campestris* has observed that all the members of the genus *Xanthomonas* are very much alike in their cultural and physiological characters and could only be differentiated from each other on the basis of their pathogenicity.

In their serological studies of the genus *Xanthomonas*, Elrod and Braun (1947) have stated that the members of *Xanthomonas* were very much alike and that, they might have been evolved from a common organism present as a saprophyte either in the soil or on the external surface of plants like the other members of colon–typhoid group. Since the morphological, cultural and physiological characters of these plant pathogens are similar to others of the colon-typhoid group, it is assumed that the plant pathogens might have the same ancestry. If that is true, we will have to accept that these organisms took different course and became parasitic to living plants by their constant association.

Link and Link (1928) are of the opinion that yellow organisms, although not alike serologically constitute a single group. The fact that certain organisms are closely related by a common host is shown in the present study also. X. *cassiae* and X. *alfalfae* which have shown closer relationship in serological studies are able to infect a common host viz. peas, which can be seen from the cross inoculation tests shown in table V. Others are serologically distinct. A closer relationship existing between X. *desmodii–gangeticii*, X. *vignicola* and X. *vesicatoria* cannot be explained at the present stage in the absence of a common host but it may be that the hosts of these organisms were growing closely in nature and that these organisms had a chance to pass from one host to another quite frequently. But

further work on this point is essential before the question of the relation between the organisms and their pathogenicity could be solved.

It will be seen from the data presented in the foregoing pages that the organisms are hardly distinguishable on their morphological and cultural behaviour. Serologically they can be grouped into 4 to 5 categories but this method will not be of much practical use in differentiating one species from another.

Results of the present studies show that the inability to liquefy Loeffler's blood serum can be made use of in separating X. *alfalfae* from the remaining seven organisms liquefying blood serum. Coons and Strong (1931) were able to divide 54 species of *Fusarium* into groups on the basis of their variation in response to growth inhibiting substance like malachite green or crystal violet added in graded dilutions to the culture medium. In the present studies, it was possible to divide the remaining six organisms into four groups, viz.

(*1*) X. *campestris*, X. *desmodii*, X. *desmodii–gangeticii*

(*2*) X. *vesicatoria*

(*3*) X. *malvacearum*

(*4*) X. *cassiae*

as their growth was inhibited at different concentrations of crystal violet. Besides these tests, inability to utilize glycine, tyrosine, guanidine hydrochloride, in presence of carbon and agglutination tests have made possible a classification of these organisms. Accordingly, a key is presented. It is to be seen by further work whether such a key could be made applicable to other leaf–spot producing organisms under study in this laboratory.

In addition, another course open is to carry out pathogenicity tests which have been rather specific although plants of the same and widely separated genera and families have heen inoculated. The assumption that they are derived from common ancestry does not lead us to any definite conclusion. Whether the organisms having one common host will change their host specificity and become one if isolated from a common host still remains to be proved.

Table VI— *Comparison of the morphological, cultural and physiological responses of some* Xanthomonas *spp.*

	Organisms									
	Xanthomonas campestris	*Xanthomonas desmodii*	*Xanthomonas desmodii gengeticii*	*Xanthomonas vignicola*	*Xanthomonas vesicatoria*	*Xanthomonas alfalfae*	*Xanthomonas badrii*	*Xanthomonas malvacearum*		
Gram stain	...	...	...	...	...	Negative	...	...	...	...	...	...
Motility	...	...	..	...	...	Motile	...	...	...	..	...	...
Gelatine	...	..	...	...	...	Liquefied	..	...	...	...	...	...
Litmus in milk	...	...	..	...	...	Reduced	...	...	..	...	...	...
Indol	...	...	...	...	...	Not formed	...	...	...	...	...	...
Starch	...	...	...	...	...	Hydrolysed	...	...	...	...	...	...
H_2S	...	...	...	...	...	Produced	...	...	...	...	...	...
Nitrate	...	...	..	..	..	Not reduced	...	...	...	...	...	...
Dextrose, Sucrose, Lactose, Glycerol	...	...	...	...	...	Acid produced	...	..	...	...	..
Citrate	...	...	...	...	...	Utilised	...	...	...	...	..	...
Inorganic Nitrogen	...	...	...	...	...	Not utilised	...	...	...	..	...
Arginine, Asparagin, Asparatic acid, Creatine, Cystine, Glycine, Guanidine-hydrochloride, Histidine, Tyrosine, Tryptophane	...	Without dextrose	...	...	No growth	...	...	...
	...	With dextrose	...	...	Good growth	...	..	..
Glutamic acid, Proteose peptone	...	Without dextrose	...	...	Good growth	...	..	....

Table VII—*Differences in cultural and serological responses of Xanthomonas spp.*

	Organisms								
	Xanthomonas campestris	*Xanthomonas desmodii*	*Xanthomonas desmodii-gangeticii*	*Xanthomonas vignicola*	*Xanthomonas cassiae*	*Xanthomonas vesicatoria*	*Xanthomonas alfalfae*	*Xanthomonas badrii*	*Xanthomonas malvacearum*
Loeffler's solidified blood serum	++++	++++	++++	++++	++++	++++	+	++++	++++
Acetic acid	-	-	-	+	-	+	-	-	-
Tartaric acid	-	-	-	-	-	-	+	-	-
Guanidine hydrochloride	+	+	+	+	+	+	+	+	+
Glycine and Tyrosine with sugar	+	+	+	+	+	+	+	+	+
Inhibition in crystal violet at	1 : 1,666	1 : 1,666	1 : 1,666	1 : 50,000	1 : 500,00	Not at 1 : 1,250	Not at 1 : 1,250	.....	1 : 16,666

Key to classify eight organisms on the basis of cultural and serological responses.

I. Non or poor liquefiers of Loeffler's blood serum.....X. *alfalfae*

II. Liquefiers.

A – Acetic acid utilised.... *X. vignicola*

B – Acetic acid not utilized.

a – *Bacteriostatic action of crystal violet*

(i) Inhibited at 1 : 50,000 *X. cassiae*

(ii) Inhibited at 1 : 16,666 *X. malvacearum*

(iii) Inhibited at 1 : 1,250 X. *vesicatoria*

(iv) Inhibited at 1 : 1,666 X. *campestris*, X. *desmodii*, X. *desmodii – gangeticii*.

b – *Serological responses*

(i) Antiserum of *X. desmodii* agglutinates X. *desmodii*

(ii) Antiserum of *X. desmodii* does not agglutinate X. *campestris*

(iii) Antiserum of X. *campestris* does not agglutinate X. *desmodii – gangeticii*

SUMMARY

Eight organisms belonging to *Xanthomonas* spp. were studied at one time for their morphology, cultural behaviour and serological relations.

All species were rod-shaped, gram negative, motile and capsulated.

All grew well in nutrient dextrose and potato dextrose agars; produced hydrogen sulphide and ammonia; liquefied gelatin; hydrolysed starch; nitrates not reduced; gave negative tests for M. R. and V. P.; non lipolytic; cellulose and citrate not utilized; liquefied blood serum; utilized egg albumen and casein; produced acid from arabinose, dextrose, maltose, lactose, sucrose but unable to utilize salicin; glycerol, mannitol and dextrin utilized, citric, acetic and tartaric acids supported differential growth; amongst the amino acids, glutamic acid only supported growth in the absence of carbon; differential growth in crystal violet medium; good growth on Patel's medium.

Agglutination test (excepting *X. badrii*) placed the organisms in five groups viz. :—

(*i*) *X. desmodii*, *X. cassiae* and *X. alfalfae*,

(*ii*) *X. campestris*,

(*iii*) *X. vignicola* and *X. vesicatoria*,

(*iv*) *X. desmodii-gangeticii* and

(*v*) *X. malvacearum*

Pisum sativum is a common host of *X. cassiae*, *X. alfalfae* and *X. badrii*. *X alfalfae* also infects *Malilotus indica* and *Trigonella foenum-graecum*. *X. vignicola* and *X vesicatoria* infect *Phaseolus vulgaris* and *Lycopersicum esculentum* respectively besides their own specific hosts.

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SELFING STUDIES WITH RACE 7 OF PUCCINIA GRAMINIS AVENAE ERIKSS AND HANN [1]

By M. K. Hingorani [2]

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Stakman, Piemeisel and Levine (1918) theorized that hybridisation of *Puccinia graminis* Pers. must take place on the barberry but did not demonstrate it. Craigie (1927, 1927, 1928) discovered the function of pycnia of stem rust and opened the way for the study of genetics in the rusts. His findings furnished a method by which controlled crossing and selfing of the rusts could be done. Waterhouse (1929) was, however, the first to report the result of a controlled cross in *Puccinia graminis.* He intermixed the nectar of pycnia of races 34 and 43 of *Puccinia graminis tritici* Erikss. and Henn. and obtained from the resulting aeciospores two races which were then unknown in Australia.

The earliest record of an artificial selfing of a known race of oat stem rust is, however, selfing of race 8 reported by Gordon and Welsh (1932). In a study of cultures arising from 16 separate aecial cups and four mass transfers of aecia, race 8 occurred 15 times, race 6 occurred 7 times, and race 7 only once. Johnson and Newton (1940) found that races 2, 7 and 10a were homozygous in nature and that races 3, 5 and 8 were heterozygous in respect to their infection types on one or the other of the three differential hosts used. From race 8 they obtained races 6 and 8 only.

This paper reports the breeding behaviour of race 7 of *Puccinia graminis avenae* through selfing. The selfing studies were expected to furnish an indication of what might happen in nature when races infect barberries and thus enable one to predict to a certain extent the pathogenic characteristics of new races that might be expected to arise in nature from new combinations.

PROCEDURE AND RESULTS

The procedure followed and the technique used in the selfing studies herein reported have been described in detail by Newton and Johnson (1932). Briefly stated, it consisted in developing teliospores of a pure culture of the race in the greenhouse, inducing them to germinate, developing aecia by mixing nectar from different pycnia, infecting seedling plants of Bond oats with the individual aecial cups and identifying the physiologic races present in the uredial cultures.

To shorten the rest period, the teliospores were frozen for a short period, then thawed and sprayed with cold tap water for a few days, thus wetting and drying them alternately. It may be described in detail as follows. Soon after the formation of teliospores they were frozen in water for 7 days in a frigidaire, running at a temperature of about −5°C. They were then thawed out and placed under

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a spray of tap water (temperature 5°–10°C.) for 7 days. After this, they were tested for infection on barberry plants. None of the cultures ever germinated at this stage. The same teliospores were then dried in the laboratory (at a low humidity) for 2 days, soaked overnight in water, and tested again for germination. This process of alternate wetting and drying (2 days wet, 2 days dry) was continued for as long as the teliospores would germinate. It might be mentioned that the criterion used for determining the germination of teliospores was the visible infection produced on barberries.

This method was successful with race 7. Teliospores started germinating after the fourth period of drying and they continued to germinate upto the tenth period of drying when the experiment was terminated.

To gain an idea of the numerical distribution of physiologic races in the progeny of selfing, aecial cups were picked off singly, at random, from numerous aecial pustules, crushed, and transferred individually to seedling leaves of a susceptible oat variety (Bond). One hundred such isolations were made, of which 95 were successful. The identification of the races was accomplished by means of the three differential varieties, Minrus, Richland and Jostrain. In the absence of definite morphological differences between races, inheritance studies were perforce limited to pathogenic characteristics which find expression in the 'infection type' on seedling leaves of cereals. The fact that an infection type of a race is relatively constant on a host variety, grown under given conditions, makes it permissible to be regarded as a character of rust.

The selfing studies carried out with race 7 indicated that race 7 was relatively homozygous with respect to factors for pathogenicity. From all the cultures, race 7 was isolated. The infection types produced by different cultures on Minrus and Jostrain ranged from 4 to 4+. On Richland, however, the infection types seemed to vary from 0; to 2, as is clear from results summarized in Table 1.

Table I—*Results of selfing studies with race 7 of Puccinia graminis avenae.*

No. of cultures isolated	Infection types recorded
25	0;
40	0; to 1
8	1
2	0; to 2
16	1 to 2
4	2

Thus it will be seen that on Richland oats some of the cultures produced infection type (0;), some produced types 1 or 2, and others produced all kinds of gradations from 0; to 2. It appears, therefore, that new combinations have

occurred on barberry plants which, although did not transcend the infection types normally produced by race 7 on the three differentials, resulted in some quantitative changes on Richland. This strongly suggests that there are probably several biotypes within race 7 which differ from one another quantitatively in their virulence on Richland. There has not yet been opportunity to test these isolates on other varieties but there is a possibility that there may be greater differences on certain other variteties than those on Richland.

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THREE NEW BACTERIAL DISEASES OF PLANTS FROM BOMBAY

By M. K. Patel, V. V. Bhatt and Y. S. Kulkarni

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Three bacterial diseases, one on *Lawsonia alba* Lam., the second on *Tamarindus indica* L. and the third on *Euphorbia pulcherrima* Willd. were observed at different places in Bombay State during 1950. On reviewing the literature and studying detailed morphological, cultural and biochemical characters, it was found that the former two are new to science while the last showed some important differences in its characters from that reported earlier by Starr and Pirone (1942). This paper is a more detailed account of a short note by the authors in 1951.

SUSCEPTS

Lawsonia alba, a small, elegant and sweet-scented bush, known as " henna" is cultivated throughout India partly for its reddish-brown or orange dye and fragrant flowers and partly as a hedge plant. The henna leaves are used for staining fingers, nails, hands and feet and for dyeing hairs.

Tamarindus indica, an evergreen leguminous woody tree growing along the road sides supplies a well known seasoning article, highly favoured by South Indians. A red dye is extracted from the leaves which contain a good amount of acid; the leaves are employed as auxiliaries in dyeing. They are believed to act as a mordant.

Euphorbia pulcherrima, an ornamental plant is probably the most important potted plant for the Christmas market because of its bright vermilion red-coloured bracts (upper leaves) contrasting with deep green coloured leaves making a colour combination pleasing to eye.

All the causal organisms were isolated separately by the usual poured plate method and their pathogenicity proved. The symptoms produced by the organisms on respective hosts in nature and by artificial inoculation are described here separately.

SYMPTOMS

The organism on *Lawsonia alba* produces few, small, water-soaked areas measuring initially 1–2 mm., mostly at the periphery of the leaf. Some of them increase in size to 3–4 mm. and become dark brown to jet black (Plate I, Fig. 1). On the other hand, undeveloped spots remain as pale brown specks, the areas around such spots turn pale brown and brittle. Bacterial ooze in the form of small, shining beads is found on both sides of the spots which get depressed on the under surface of the leaves, the corresponding areas on the upper surface being raised and presenting a pale white appearance due to bacterial exudation. The pathogen infects the leaves only.

The organism on *Tamarindus indica* produces on the leaflets few, small, water-soaked spots measuring initially 0·5–1 mm. after an incubation period of

EXPLANATION OF PLATE

Fig. 1. Dark brown to jet black, necrotic spots on leaves of *Lawsonia alba*.

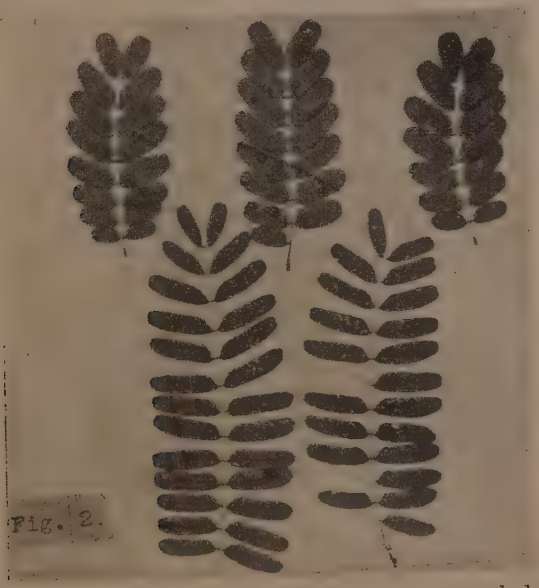

Fig. 2. Dark brown to jet black, necrotic spots surrounded by small halo on leaves of *Tamarindus indica*.

Fig. 3. Leaves of *Euphorbia pulcherrima* showing angular, dark spots and visibly clear water soaked area on lower surface with pearly beads.

Fig. 4. Water-soaked cankerous streaks on stem of *Euphorbia pulcherrima* producing cracking.

13–15 days. In the beginning, the spots are pale brown with a small yellow halo around them which later increase in size to 3–5 mm., become irregular and dark-brown to jet black (Plate I, Fig. 2). Spots also develop along the line of mid-vein but the pathogen does not become vascular. Deep black spots on rachis and petiole are rarely seen. Bacterial ooze in the form of fine scales or small shining beads is found on both sides of spot which is raised due to heavy bacterial exudation. The pathogen infects leaves only.

The organism on *Euphorbia pulcherrima* produces on the leaves few to numerous, small, water-soaked spots visibly clear on the lower surface of the leaf measuring initially 0.5 mm., fairly well distributed all over the surface. With the progress of the disease, the spots increase in size to 1.5 mm., become angular and brown with deep coloured periphery (Plate I, Fig. 3). Like other bacterial diseases, the ooze in the form of small, shining, pearly beads is found on the under surface of leaves.

Tender stems when infected artificially by rubbing show small, vertical water-soaked streaks which in due course develop into canker and cracking on the stem (Plate I, Fig. 4). In the rainy season, these streaks proceed on both directions. In no case did spraying alone produce streaks and the pathogen never migrated to stem from leaf through the petiole or *vice virsa*. Though the symptoms of the disease agree with those reported by Starr and Pirone (1942) on the same host in some respects, systemic infection could not be traced. It was also interesting to note that the bracts were not infected.

MORPHOLOGY

Morphology and staining reactions of these organisms which are being designated *Xanthomonas lawsoniae*, *Xanthomonas tamarindi* and *Xanthomonas poinsettiaecola* are presented in Table I from which it can be easily seen that they are indistinguishable from one another.

Table I—*Morphology of the organisms*

Morphology	*Xanthomonas lawsoniae*	*Xanthomonas tamarindi*	*Xanthomonas poinsettiaecola*
Shape	Rods, mostly single, or rarely in chains.	Rods, single	Rods, in chains of 2-3
Size	0.7-1.8 μ	0.6-1.6 μ	0.6-1.3 μ
Gram reaction	.....	Negative	.....
Motility	.....	Single polar flagellum	.....
Capsule	.....	Capsulated	.....
Spore	.....	No	.....
Acid fast	.....	Not	.....

CULTURAL CHARACTERS

On potato dextrose agar (P. D. A.) plates, all the cultures produced circular, entire, smooth, shining, convex, yellow colonies with striations at the

periphery. On potato dextrose agar slants, copious, raised and filiform growth was observed. Potato cylinders turned grey in 7 days with the growth covering the entire surface of the cylinder. On nutrient agar (N. A.), colonies were flat, circular and shining. In Table II are given the colony colour and size of each after 5 days' growth at 31°C.

Table II—*Colony size and colour.*

Colony	*Xanthomonas lawsoniae*		*Xanthomonas tamarindi*		*Xanthomonas poinsettiaecola*	
	P. D. A.	N. A.	P. D. A.	N. A.	P. D. A.	N. A.
Size	18 mm.	6–8 mm.	14 mm.	5–6 mm.	20 mm.	12 mm.
Colour	Empire yellow	Pale lemon yellow	Martius yellow	Picric yellow	Picric yellow	Pale lemon yellow

All the cultures made good growth on yeast–chalk–glucose agar slants and remained viable and pathogenic for at least 4 months, a finding in conformity with Dowson's (1949) for *Xanthomonas* spp. Optimum temperature for growth was between 27°–31°C. and the thermal death point about 51°C.

BIOCHEMICAL REACTIONS

Unless otherwise mentioned, all the media and their corresponding tests were carried out according to the methods recommended in the manual (1944) and the readings recorded after 7 days at 31°C.

All the cultures made good growth in synthetic media such as Cohn's, Fermi's and Uschinsky's turning the last named alkaline. Litmus milk was changed from mauve (R) to pink in 3–4 days and peptonised completely in 7–10 days, *Xanthomonas lawsoniae* being slow in this reaction. All liquefied gelatin and hydrolysed starch and casein. Hydrogen sulphide and ammonia were produced by all from 1 per cent proteose peptone and peptone water respectively. Indol was not produced from tryptophane. All were M. R. and V. P. negative. None produced nitrite from peptone–beef nitrate but in modified Richard's medium, all but *Xanthomonas tamarindi* made good growth and produced nitrite. None could grow in Koser's uric acid medium but good, whitish, entire, circular colonies, changing the medium alkaline were observed on Simmon's citrate agar plates. Only *Xanthomonas tamarindi* liquefied Loeffler's solidified blood serum in 18 days. All tolerated sodium chloride up to 3 per cent in synthetic carbohydrate medium and grew best in neutral medium but tolerated pH ranging from 3 to 9.

FERMENTATION OF CARBOHYDRATES

A synthetic medium containing 1 per cent carbon compounds or 0.1 per cent organic acids was prepared, neutralised and tubes containing 5 ml, of this medium were sterilised for 3 successive days in the Arnold steriliser. Tubes were

inoculated with a loop drawn from a liquid culture and readings taken after 8 days' incubation showed that all produced good growth and acid in arabinose, galactose, levulose, dextrose, lactose, maltose, sucrose, dextrin, glycerol, dulcitol and mannitol. They produced some growth and slight acid in raffinose, good growth and alkali in acetic and citric acids and slight growth in tartaric acid with no change in pH of the medium. There was no growth in salicin and oxalic acid. Carbon metabolism of *Xanthomonas poinsettiaecola* when studied in a medium containing 0.3 per cent yeast extract, 0.5 per cent peptone and 1 per cent sugars mentioned above produced alkali in all the sugars except in maltose, because the acid produced by the fermentation of carbohydrates was neutralised by ammonia produced from the break-down of peptone, the extra production of ammonia from peptone changing the medium alkaline. The growth in yeast-peptone medium containing salicin as against no growth in synthetic medium with salicin clearly shows that peptone supplies both nitrogen and carbon for growth.

UTILISATOIN OF INORGANIC NITROGEN

Modified Richard's medium with 1 per cent dextrose and 0.14 per cent of inorganic nitrogen as suggested by Patel and Kulkarni (1949) in place of 1 per cent potassium nitrate was prepared, neutralised and sterilised for 3 successive days in Arnold and inoculated. Results taken after 8 days' incubation are given in Table III.

Table III—*Utilisation of inorganic nitrogen*

Inorganic Nitrogenous salts	*Xanthomonas lawsoniae*	*Xanthomonas tamarindi*	*Xanthomonas poinsettiaecola*
Ammonium citrate	4*	4	4
,, nitrate	4	4	4
,, phosphate (Monobasic)	4	4	4
Ammonium sulphate	4	4	4
Calcium nitrate	2	0	2
Magnesium nitrate	2	0	2
Potassium nitrate	3	1	4
Sodium nitrate	3	1	4

* 0 = No growth, 1 = Slight, 2 = Fair, 3 = Good, 4 = Heavy growth

It is clear from the above table that *Xanthomonas lawsoniae* and *Xanthomonas poinsettiaecola* utilise all the inorganic nitrogenous salts fairly well while *Xanthomonas tamarindi* does not utilise them to the same extent except ammoniacal salts.

UTILISATION OF ORGANIC NITROGEN

Medium as advocated by Mushin (1938) was prepared with and without dextrose to which different amino acids were added. All utilised glutamic acid and proteose peptone without dextrose while addition of dextrose was necessary

for good growth in the case of arginine, asparagine, aspartic acid, creatine, glycine, guanidine hydrochloride, histidine, tryptophane and tyrosine.

HOST RANGE

From the morphological, cultural and biochemical characters so far studied, it is beyond doubt that the organisms under study belong to the genus *Xanthomonas*. Wernham (1948) and Patel, Dhande and Kulkarni (1951) reported that in the genus *Xanthomonas*, host specificity is the only reliable character in establishing a species. It was, therefore, thought worthwhile to cross-inoculate each of the 3 organisms against 21 suscepts known to be affected by 18 *Xanthomonas* spp. described from this laboratory and a few more plants of the same family to which the suscepts of the 3 organisms belong. Plants were grown in sterilised soil in pots and inoculated when a month old. A separate atomiser was used for each organism and plants infected with organism were kept separate. Punctures with sterile needles were also made on the leaves and tender stems before inoculation. The plants used for cross inoculation trials were :—*Acalypha* sp., *Begonia* sp., *Brassica oleracea*, *Caesalpinia sepiaria*, *Cajanus cajan*, *Capsicum annum*, *Cassia tora*, *Clerodendron phlomoides*, *Croton* spp., *Desmodium diffusum*, *Desmodium gangeticum*, *Dolichos lablab*, *Euphorbia geniculata*, *Euphorbia hypericimia*, *Euphorbia milli*, *Euphorbia pulcherrima*, *Gossypium herbaceum*, *Lagerstroemia indica*, *Lagerstroemia villosa*, *Lawsonia alba*, *Manihot esculenta*, *Medicago sativa*, *Pisum sativum*, *Poinciana regia*, *Punica granatum*, *Ricinus communis*, *Sesbania aegyptiaca*, *Stizolobium deeringianum*, *Tamarindus indica*, *Trigonella foenum-graecum*, *Vigna sinensis*, *Woodfordia floribunda* and *Xanthium strumarium*. The inoculation trials made at 5 different times showed conclusively that the organisms are restricted to the suscepts from which they were originally isolated. *Xanthomonas tamarindi*, however, does show mild infection of *Caesalpinia sepiaria* after a long period of incubation.

TAXONOMY AND NOMENCLATURE

The morphological, cultural and physiogical characters of *Xanthomonas poinsettiaecola* seem quite different in some respects from those of *Corynebacterium poinsettiae* as reported by Starr and Pirone (1942). A comparative statement giving the distinguishing characters of the two organisms is, therefore, presented in Table IV.

It is evident from Table IV that *C. poinsettiae* differs from *Xanthomonas poinsettiaecola* in 3 important characters, viz. Gram reaction, production of yellow pigment on solid media and carbon metabolism. *Xanthomonas poinsettiaecola* produces strong acid from lactose but fails to grow in salicin-characters typical of *Xanthomonas*. Besides, *Xanthomonas poinsettiaecola* does not produce wilt.

It was proposed to assign each a specific name as the organisms are new to science :—*Xanthomonas lawsoniae* from *Lawsonia alba*, *Xanthomonas tamarindi*

Table IV—*Comparison of Corynebacterium poinsettiae with Xanthomonas poinsettiaecola*

Tests	*Corynebacterium poinsettiae*	*Xanthomonas poinsettiaecola*
MORPHOLOGY AND STAINING REACTIONS		
1. Shape	Varying shape like rods, curved, coccoid, clavate etc.	Straight slender rods only.
2. Motility	Motile by one, rarely two, pol r or lateral flagellum, earlier reported as non-motile.	Motile by one polar flagellum.
3. Gram reaction	Gram positive becoming Gram variable.	Gram negative only.
CULTURAL CHARACTERS		
1. Beef extract agar	Colonies slightly convex, colourless almost transparent.	Colonies flat, pale yellow.
2. Potato dextrose agar	Colonies salmoned colour becoming ochraceous salmon.	Colonies picric yellow.
3. Loeffler's blood serum	Liquefied in 3 to 10 days.	No liquefaction in 20 days.
4. Litmus milk	Slight acid or no visible change for 1 to 2 weeks.	Fair acidity in 6 to 7 days, medium changed to pink.
CARBON METABOLISM		
Sugar fermentation	Yeast-peptone medium	Yeast-peptone medi um / Synthetic medium
(a) Mannitol	No acid	Alkali / Acid
(b) Dulcitol	No acid	Alkali / Acid
(c) Arabinose	Weak or slow acid production	Alkali / Acid
(d) Lactose	Weak or slow acid production	Alkali / Acid
(e) Glucose	Moderate and abundant acid	Alkali / Acid
(f) Galactose	Moderate and abundant acid	Alkali / Acid
(g) Sucrose	Moderate and abundant acid	Alkali / Acid
(h) Maltose	Moderate and abundant acid	Sl. acid / Acid
(i) Glycerol	Moderate and abundant acid	Alkali / Acid
(j) Raffiniose	Moderate and abundant acid	Alkali / Sl. acid
(k) Salcin	Moderate and abundant acid	Alkali / No growth

from *Tamarindus indica* and *Xanthomonas poinsettiaecola* from *Euphorbia pulcherrima*. The technical description of the 3 organisms in respect of important and common characters is given below:—

Short rods; single polar flagellum; Gram negative; capsulated; on potato dextrose agar plates, colonies are circular, with entire margins, smooth, shining, convex with striations at the periphery only, colour yellow; gelatin liquefied; starch and casein hydrolysed; milk peptonised and litmus milk turned pink; hydrogen sulphide and ammonia produced from peptone; nitrites produced by all except *Xanthomonas tamarindi*; M. R. and V. P. tests negative; acid but no gas from dextrose, lactose, maltose and sucrose; no growth in salicin; optimum temperature for growth 27°–31°C; thermal death point about 51°C.

SUMMARY

Three plant bacterial pathogens viz. *Xanthomonas lawsoniae*, *Xanthomonas tamarindi* and *Xanthomonas poinsettiaecola* causing leaf spots on *Lawsonia alba*, *Tamarindus indica* and *Euphorbia pulcherrima* respectively have been isolated and described.

The symptoms of the disease on each plant and the morphological, cultural and physiological characters of each pathogen have been described. Each pathogen shows suscept specificity.

Diseased specimens of leaves of each host are deposited in the herbaria of the Plant Pathologist to Government of Bombay, College of Agriculture, Poona., Indian Agricultural Research Institute, New Delhi., and of the Commonwealth Mycological Institute, Kew, England.

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FUNCTION OF SPORE MATRIX IN COLLETOTRICHUM LINDEMUTHIANUM *

By R. S. Mathur

(Accepted for publication, October 18, 1951)

According to Edgerton (1910), the mucilagenous substance in which the spores of *Colletotrichum lindemuthianum* are embedded helps in preserving the viability of the spores. During wet weather this gel-like matrix absorbs water and facilitates the dissemination of spores. Recently Mathur *et al* (1950) reported that spores with matrix, used as inoculum, stimulated greater sporulation in the *gamma* strain of the fungus than did spores washed free of matrix. Perhaps the matrix served a nutritive function, namely that of stimulating better production of spores. This possibility was tested in the following experiments and compared with the effects of vitamins, inositol and biotin, and plant extracts from bean and pea seeds which normally favour heavy sporulation.

MATERIALS AND METHODS

A single spore culture from an isolate of the *gamma* strain of *Colletotrichum lindemuthianum* was obtained from the Department of Plant Pathology, Cornell University and used throughout the investigation. Glucose—neopeptone medium† was used for obtaining abundant sporulation. When nonsporulating colonies of the fungus were required, the same medium was used after boiling it for a few minutes with 5 gm. of norit (activated charcoal). A concentrated suspension of conidia and matrix was obtained by soaking the heavily sporulating colonies for a few days in double distilled water, then gently scraping the top layer of spores with a sterilised cover glass. This suspension was centrifuged several times at 1000 revolutions per minute until a clear solution of the matrix was decanted and filtered aseptically through a Morton filter (1944). The master solutions of inositol contained 5 mgm. of inositol per ml. and 5 mgm. of biotin per ml. The pea and bean extracts were prepared by chopping 50 pea or bean seeds in 125 c. c. of double distilled water in a Waring blender (1946) and then centrifuging at 500 revolutions per minute and finally filtering the extract in a Morton filter. All test solutions were stored at 4°C. until required for use.

EXPERIMENTAL

Three experiments were designed in order to compare the spore producing capacities of matrix solution, inositol, biotin, and bean and pea extracts.

In the first experiment, circular discs about 7 mm. in diameter were cut out aseptically from 10 day old non-sporulating colonies of *Colletotrichum lindemuthianum*. Ten such discs were soaked in each of the test solutions for 24 hours

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* Condensed from a portion of a thesis, submitted to the Graduate Faculty of the University of West Virginia, Morgan town, W. Va. U. S. A. in partial fulfillment of the degree of Doctor of Philosophy.

† The glucose—neopeptone medium contained glucose, 2.8 gm , $MgSO_4$ $7H_2O$, 1.23 gm. KH_2PO_4, 2.72 gm., neopeptone (Difco), 2.0 gm., agar: 20.0 gm., and double distilled water to make 1000 ml.

at 4°C. The master solutions of inositol and biotin were used in one fifth concentration. Under identical conditions an equal number of mycelial discs were soaked in double distilled water to serve as controls. After treatment, the discs were planted on 2% pyridine purified water agar in Petridishes which were incubated at 20°C.

In the second experiment, Penicylinders were planted at the growing edges of the non-sporulating colonies of the fungus. In each Petridish 4 Penicylinders were planted of which two Penicylinders contained 4 drops of the test solutions and the other two had 4 drops of distilled water to serve as control. Each treatment was replicated four times and all the plates were incubated at 20°C.

In the third experiment, one drop was taken from the master solutions of inositol or biotin and mixed with separate lots of 5 c. c. double distilled water. Five drops from these solutions were poured separately over 10 day old colonies of the fungus. Other non—sporulating colonies of the same age were similarly treated with 5 c. c. of bean or pea extracts or with an equal amount of double distilled water. Each treatment was replicated four times and the plates incubated as usual at 20°C.

In every experiment, the acervuli and spores developed after the fourth day. Final observations were taken on the seventh day. All the experiments were repeated thrice and each time almost similar results were obtained. The combined data from these experiments are given in the following table.

Table 1— *The effect of matrix, vitamins and plant extracts on the sporulation of the* gamma *strain of* Colletotrichum lindemuthianum *at 20°C.*

Treatment	No. of acervuli per disc	No. of acervuli per Penicylinder area	No. of acervuli per plate
Matrix solution	6	3	9
Inositol	4	—	11
Biotin	4	—	11
Bean extract	20	3	11
Pea extract	15	5	—
Distilled water	0	0	0

All experiments lead to the same conclusions regarding the beneficial effects of spore matrix on spore production, but more significant data were obtained in the first and the third experiments. In the first experiment, soaking of mycelial discs in bean and pea extracts stimulated four to five times greater production of acervuli than soaking in inositol, biotin or matrix solutions. Comparatively few acervuli were formed in the second experiment where stimulation for acervulus production was better with the pea extract than with the bean extract or the

matrix solution. In the third experiment, all treatments were almost equally effective in an abundant production of the acervuli. It is significant that no acervuli were produced in the control plates in any experiment.

DISCUSSION

The stimulation to conidial formation in *Colletotrichum lindemuthianum* by matrix is an observation not recorded earlier for any other fungus. It has been found that the fungus is partially deficient to inositol and biotin (Mathur *et al* 1949) and although the matrix solution was not bio-assayed for vitamins, the similarity in the stimulation of spore production by matrix and the vitamins suggests that the matrix contains certain active substance. The vitamin deficient mycelium was exposed to aqueous solutions of the test substances in all the experiments. This procedure of witholding the nutrients and increasing the concentration of vitamins allowed the reserve materials in the mycelium to be utilised for sporulation. The mycelium contained the same reserve of food, but the addition of sufficient quantities of inositol and biotin to which the fungus was partially deficient, catalysed the processes which lead to sporulation. Since the effect of matrix was similar to that of inositol and biotin, a portion of the activity of the matrix solution can be ascribed with a high probability to the presence of these vitamins in the matrix. The possibility of other active substances in the matrix cannot, however, be excluded.

The extracts from pea and bean seeds supported the maximum sporulation. This can be ascribed to the presence of both vitamins and nutrients in the extracts. Probably the addition of vitamin-free nutrients would favour more growth than sporulation, but such a possibility was not tested in this investigation. Leonian (1936) reported that pea extract was specific for the sexual reproduction of many species belonging to the genera *Pythium* and *Phytophthora*. It was found that this substance stimulated the asexual reproduction of *Colletotrichum lindemuthianum* also. Leach (1923) observed the best sporulation of this fungus on bean agar. He also reported considerable increase in the germination percentage of the conidia in the presence of bean pods. Obviously bean extract even in very minute doses stimulated the germination of conidia.

In nature, the bean anthracnose disease spreads rapidly when splashes of rain wash away the spores. When a rain drop falls on the anthracnose lesions of beans, the mucilagenous matrix swells up and gradually dissolves in water which also contains minute quantities of the bean extract. The disseminated spores carry with them minute quantities of matrix and the bean extract in which they first germinate profusely. Later, the new mycelium sporulates heavily under stimulus from the matrix. In the light of the experiments described elsewhere there is circumstantial evidence to show that the formation of conidia *Colletotrichum lindemuthianum* within the mucilagenous matrix, is probably the nature's device to stimulate better production of spores at each successive generation and thus facilitate the survival of the species. Whether spore matrix plays a similar role in other fungi cannot be stated and has to be investigated for individual species.

SUMMARY

The stimulation of asexual reproduction in the *gamma* strain of *Colletotrichum lindemuthianum* by the spore matrix is a function of the matrix not recorded previously for any other fungus. Perhaps in this manner the matrix helps in the natural spread of the bean anthracnose disease.

The spore producing capacities of the spore matrix, vitamins and extracts from bean and pea seeds have been compared.

Thanks are due to Dr. J. G. Leach who suggested the problem and to Drs. V. G. Lilly and H. L. Barnett of the West Virginia University for many valuable suggestions. Thanks ars also due to Dr. R. S. Vasudeva, Head of the Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi for critically going through the manuscript.

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DAMPING OFF OF TOMATO SEEDLINGS—ITS CAUSE AND CONTROL

By M. L. Gattani & T. N. Kaul

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Damping-off of tomato seedlings occurs very frequently in seed beds; the disease usually appearing in two phases. In the pre-emergence phase, the young seedling is killed before it reaches the surface of the soil and in the post-emergence phase the disease is characterized by toppling over of the infected seedlings at any time after their emergence from the ground, until stems have hardened enough to resist the invasion. During July 1948, the disease was seen in a severe form in the nursery of the Botany Division, I. A. R. I., New Delhi. Investigations on this disease were, therefore, undertaken in the Mycology Division, I. A. R. I. to determine the cause and devise some effective control measure.

MATERIALS AND METHOD

The fungus was isolated from the diseased seedlings brought from the nursery of the Botany Division. For this purpose, portions of the hypocotyl were surface sterilized for one minute in 0. 01 percent solution of mercuric chloride and then plated out on potato dextrose agar. The culture thus obtained was purified by single hyphal tip isolations. Inoculum was raised in 7 cm. quaker oat agar plates, one petri plate being used for infesting one 4 inch pot. The field soil used for the work was sterilized at 15 lbs. pressure for four hours. For pathogenicity tests, seeds of the variety "Suttons early market" from which the organism was isolated were used.

LITERATURE REVIEW

Middleton (1943) has quoted ten different species of *Pythium*—*Pythium adhaerens, Pythium aphanidermatum, Pythium artotragus, Pythinm debaryanum Pythium megalacanthum, Pythium myriotylum, Pythium perniciosum, Pythium salpingophorum, Pythium spinosum,* and *Pythium ultimum* parasitizing tomatoes in different countries. Ramkrishna Ayyar (1929) reported from India that *Pythium aphanidermatum* isolated by him from *Opuntia dillenii* successfully infected tomato. Berkley (1925), Weber and Ramsey (1926) and Person and Chilton (1931) attributed *Pythium debaryanum* as the causal agent of damping off of tomato seedlings. *Pythium ultimum* was considered as the causal organism of damping off of tomato seedlings by Alexander *et al* (1931) and Horsfall (1880).

The control of damping off of tomato seedlings by cultural modifications was first suggested by Atkinson (1895). Brien and Chamberlain (1936) suggested soil sterilization by steam for its control. Formaldehyde drench, modified soil treatment with formaldehyde and treatment of soil with formaldehyde dust have also been recommended for the control of damping off. Humbert (1918) reported that spraying of beds with Bordeaux mixture controlled the post-emergence phase of the damping-off of tomato seedlings.

Treatment of seeds with fungicides has also been suggested for the control of damping-off. Horsfall (1930, 1932, 1933 and 1934) recommended seed treatment with red oxide of copper, copper sulphate monohydrate and copper sulphate solution. Chamberlain and Brein (1937) suggested Ceresan U. T. 1875, Agrosan G, and copper carbonate for the treatment of seed. Doolittle (1944) obtained significant better stands in tomato with yellow cuprocide, Arasan, Spergon, New Improved Ceresan, and copper sulphate soak. Combination of seed treatment with red oxide and soil treatment with zinc oxide has been recommended by Horsfall (1934) for the effective control of damping off of tomato seedlings.

EXPERIMENTAL

The damped off plants on microscopic examination revealed the presence of abundant, non septate, fungus mycelium in discolored cortical tissue. Numerous isolations from these plants invariably yield *Pythium* sp. It was established that the fungus caused pre-emergence as well as post-emergene damping off of tomato seedlings and could be reisolated from damped off plants.

To determine the identity of the species, its cultural characters were studied on plain agar, potato dextrose agar, Quaker oats agar and corn meal agar. On plain agar the growth was submerged and very scanty. A fluffy aerial growth was formed on potato dextrose agar but on this medium oospores and lobulate sporangia were scanty. On oat meal agar and corn meal agar the growth was luxuriant with plenty of oospores and sporangia. Cultures from single hyphal tip isolations from the fungus always bore oogonia and antheridia, thus establishing the homothallic nature of the fungus.

MORPHOLOGY

Mycelium irregularly branched, coenocytic when young, the filaments being full of granular protoplasm. In older cultures the filaments were hyaline and septate, hyphae 2.8 to 7.2 μ mostly from 3.6 to 5.4 μ in diameter. Sporangia from 84.0 to 147.0 μ long and 8.5 to 12.5 μ broad; zoospores reniform, biciliate and produced in a vesicle, the number being 5 to 25 per vesicle. Oogonia smooth walled, terminal and spherical measuring 17 to 25.5 μ in diameter. Antheridia mostly intercalary, occasionally terminal, short and cylindric, 1 to 2 per oogonium, both monoclinous and diclinous measuring 8.0 to 12.5 x 10.5 μ to 14.0 μ. Oospores aplerotic, smooth, thickwalled and 12.5 to 21.0 μ in diameter.

For determiniug the temperature relations of the fungus triplicate plates of potato dextrose agar after uniform seeding were kept at 5, 10, 21, 26, 33 and 37°C. Growth of the fungus was recorded every 24 hours by measuriug the vertical and horizontal mean diameter of the colony. The fungus grew well from 26 to 37°C, the optimum being 33°C. At 5°C the fungus did not grow even after 10 days but at 10°C there was good growth after 8 days. It was, therefore, concluded that the minimum and the optimum temperatures for the growth of the fungus were about 10 and 33°C respectively.

On the basis of morphological and cultural characters, growth habit and temperature relations the fungus was identified as *Pythium aphanidermatum* (Edson) Fitz.

CONTROL

Experiments were conducted to test the relative efficacy of seed treatment with red oxide of copper, copper sulphate dust, Ceresan and copper sulphate soak as these treatments have been reported to give satisfactory control of damping off of tomato seedlings. The dosage of the fungicide for treating the seed was as follows:-

Red oxide of copper—One and one half ounce per pound of seed (1932).
Copper sulphate dust— ,, ,, ,, ,, ,, ,, ,, ,, ,, (1932).
Ceresan — 0. 2 ounce ,, ,, ,, ,, (1937).
Copper sulphate soak— The seeds were dipped in 10 percent solution for one hour as recommended by Alexander *et al* (1931).

Four pots each containing 50 seeds sown in infested soil were used for each treatment. The control consisted of two sets-one with infested and the other with uninfested soil. In both sets the seeds did not receive any treatment. After emergence of the seedlings, readings were frequently taken for three to four weeks and the percentage of pre-emergence damping off was calculated on the basis of the total number of seedlings emerged in uninfested control pots. The experiment was conducted in three series, the planting in each series being made after 10 day interval. The results of the three separate trials are presented in Table 1.

Table 1—*Relative efficacy of different seed treatments for the control of damping off of tomato seedlings*

Seeds Treatment	Series	No. of seeds sown	No. of seedlings emerged	No. of plants damped off		Percent of plants damped off	Average
				Pre emergenc	Post emergence		
Control	1	200	168	—	—	—	—
	2	200	140	—	—	—	—
	3	200	128	—	—	—	—
Control (Infested soil)	1	200	68	100	24	73.8	—
	2	200	48	92	8	71.4	72.3
	3	200	52	76	16	71.8	—
Copper sulphate soak	1	200	88	80	4	50.0	—
	2	200	88	52	4	40.0	42.5
	3	200	88	40	8	37.5	—
Red oxide of copper	1	200	72	96	16	66.6	—
	2	200	68	72	8	57.1	62.0
	3	200	60	68	12	62.5	—
Ceresan	1	200	80	88	20	64.2	—
	2	200	60	80	8	62.8	59.0
	3	200	72	56	8	50.0	—
Copper sulphate dust	1	200	76	92	20	66.6	—
	2	200	56	84	4	62.8	61.8
	3	200	68	60	12	56.2	—

It would be seen from Table 1 that none of the treatments gave complete control of the disease. The seed lots which were treated with 10 percent copper sulphate soak for one hour showed 42.5 percent damping-off where as the control for the same gave 72.3 percent damping-off. Red oxide of copper, Ceresan, and copper sulphate dust did not prove as effective as copper sulphate soak in the partial control of damping-off.

CONTROL OF DAMPING OFF BY SOIL DISINFECTION

Disinfection of soil with formaldehyde has been reported to give satisfactory control of damping-off. Experiments were, therefore, performed by the writers to control damping-off of tomato seedlings by disinfecting the soil with formaldehyde. Disinfection of the soil was carried out according to the method recommended by Sherbakoff *et al* (1943). For this purpose 10 ml. of commercial formalin was diluted with 50 ml. of distilled water and the solution so obtained was sprinkled over a 20″ x 14″ x 2.75″ flat of soil which had been previously infested with the damping-off fungus. The soil after treatment was thoroughly raked and allowed to stand for 24 hours. It was then filled in 4″ pots each of which contained fifty untreated seeds. The control consisted of two sets- one with infested soil and the other with uninfested soil. The results are presented in Table 2.

Table 2—*Control of damping-off by soil disinfection*

Soil Treatment	Series	Total no. of seeds sown	No. of seedlings emerged	No. of plants damped off		Percent of plant damped off	Average
				Pre emergence	Post emergence		
Control (uninfested Soil)	1	200	148	—	—	—	—
	2	200	160	—	—	—	—
Control (infested soil)	1	200	72	76	24	67.4	69.9
	2	200	80	80	36	72.5	
Infested soil treated with formal-dehyde	1	200	144	4	12	10.8	12.9
	2	200	152	8	16	15.0	

It would be seen from the accompanying table that in the infested soil which had been disinfected with formaldehyde, damping-off of tomato seedlings was reduced from 69.9 percent in the control to 12.9 percent.

EXPERIMENT ON THE CONTROL OF DAMPING-OFF BY COMBINED SEED AND SOIL TREATMENT

Horsfall (1934) recommended a combined treatment of seeds with red oxide of copper and soil with zinc oxide for the effective control of damping-off of tomato seedlings. In our experiments soaking of the seeds with 10 percent copper sulphate solutions gave the best results among all the seed disinfectants.

An experiment was, therefore, set to investigate the efficacy of combined treatment of the seed with 10 per cent copper sulphate solution and soil with formaldehyde. The results are incorporated in Table 3.

Table 3—*Control of damping-off by combined seed and soil treatment*

Treatment	Series	Total no. of seeds sown	No. of seedlings emerged	No. of plants damped off		Percent plants damped off	Average
				Pre emergence	Post emergence		
Control	1	200	164	—	—	—	—
	2	200	160	—	—	—	—
Infested check	1	200	80	84	32	70.7	70.7
	2	200	84	76	36	70.7	
Seed treatment with copper sulphate and soil treatment with HCHO	1	200	156	8	4	7.3	7.4
	2	200	148	12	0	7.5	

It would be seen from table 3 that combined seed and soil treatment reduced the percentage of damped off seedlings from 70.3 per cent in the infested check to 7. 4 per cent. It, therefore, gave a better control of the disease than was the case in the experiment on seed treatment alone or with soil treatment alone.

SUMMARY

1. *Pythium aphanidermatum* (Eds.) Fitz. has been found to cause pre-emergence and post-emergenc damping-off of tomato seedlings.

2. Dusting the seeds with red oxide of copper, copper sulphate dust, Ceresan and soaking the seed in 10 per cent copper sulphate solution reduced the incidence of damping-off by 10, 11, 13 and 29 per cent respectively.

3. Soil disinfection with formaldehyde reduced the incidence of damping-off by 57 per cent.

4. A combination of soil treatment with formaldehyde and seed treatment with 10 per cent copper sulphate solution for one hour reduced the incidence of damping-off by 63 per cent.

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SEEDLING BLIGHT AND FOOT ROT OF CEREALS CAUSED BY FUSARIUM AVENACEUM (FR.) SACC. AND FUSARIUM CULMORUM (W. G. SM.) SACC.

By B. K. Bakshi

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In the spring of 1947, I encountered failure of a barley crop on a farm in North Clermiston, Barnton, near Edinburgh. The stand was thin due to pre-emergence and seedling blight. The bases of seedlings above the ground showed dark-brown lesions and many plants were dying in the 3–4 leaf stage. Isolations from the bases of diseased seedlings constantly yielded *Fusarium avenaceum* and *Fusarium culmorum* in culture, and failure of the crop was ascribed to attack by these two fungi. Pathogenicity experiments of the two isolates were carried out on seedlings in greenhouses.

Healthy seeds were soaked in water for 24 hours, surface sterilized in 0.1% mercuric chloride for two minutes and later washed in several changes of sterile water. The experiments were conducted in ordinary 6 inch clay pots. The agar-disc method (Garrett, 1936) was used for inoculating the grains. Seeds were planted at 1 in. depth. Experiments were carried out in greenhouses at a temperature of 40°–45°F. For each treatment, the seeds were inoculated by the fungus and to compare emergence between infected and control series an equal number of seeds was sown without any fungus inoculum. The seedlings were harvested and examined after four weeks. The particular pathogen was always re-isolated from diseased seedling after they were harvested. The seedlings in the infected series were placed into one of the five following ratings:—

0. Healthy seedlings.
1. Slight basal lesions.
2. Moderate basal lesions.
3. Severe basal lesions.
4. Seedling blight, where the seedlings die soon after emergence.

Each experiment has been analysed in two parts. In the first, the emerged seeds in the infected and control series have been compared to determine whether the rates of emergence vary. In the second, the emerged seedlings in the inoculated series have been put under different ratings and the treatments analysed. They are dealt in subsections (a) and (b) respectively in each of the tables 1–4.

GREENHOUSE EXPERIMENTS

Pathogenicity tests on six varieties of barley:—The tests were carried out in sterile sand. 25 seeds were planted in each pot. For each variety 50 seeds were infected with *F. avenaceum* (treatment A), an equal number with *F. culmorum* (treatment B) and the same number was used as control (treatment C). This terminology is used throughout.

The number of seedlings which emerged in each of the three treatments is given in table 1 (a). It also gives the results of X^2 (chisquare) tests applied to test the significance in differences in the rates of emergence among the three treatments for each variety and the varietal differences within each treatment.

Table 1 † (a)—*Emergence of six varieties of barley under treatments A, B, and C in sterilized sand.*

Veriety of barley	Treatments			X^2_2	X^2_2		
	A	B	C		C vs. A	C vs. B	A vs. B
	Emergence out of 50						
Spratt archer	27	37	41	9.90**	9.01**	0.93	4.34*
Pioneer	19	41	42	31.07***	22.24***	0.07	20.17***
Prefect	17	38	42	31.57***	25.84***	1.00	17.82***
Plumage archer	17	41	41	34.22***	23.65***	0.00	23.65***
Abed maja	16	39	45	42.18***	35.35***	2.68	21.37***
Rigel	14	33	40	29.72***	27.21***	2.49	14.49***
X^2_5	8.90	4.96	2.17				

The emergence in treatment A is significantly less than in B and C and there is no significant difference between the last two for any variety, but emergence in the seeds infected with *F. culmorum* is less than the emergence in the control for each variety. This is some indication that *F. culmorum* produces a slight pre-emergence blight, which is not statistically significant for each variety separately, but when the results of all the varieties are pooled the emergences total 229 and 251 out of 300 each for treatments B and C respectively, and these are significantly different at the 5% level of probability.

† The suffix attached to X^2 in this and other tables represents degrees of freedom.

* Significant at 5% level of probability.

** „ „ 1% „ „ „

*** „ „ 0.1% „ „ „

The results of X^2 tests given in the last row of table (a) show that varietal differences in emergence are not significant for each treatment. The non-significant X^2 for treatment C proves that the viability of seeds was similar for all varieties. It is therefore valid to compare the emergence among the varieties for treatments A and B. Although the X^2 test shows that differences among the six varieties are not significant, Spratt archer seems to be significantly different from the remaining five varieties in its resistance to *F. avenaceum* as regards pre-emergence blight.

The number of emerged seeds under the five rating classes (0 to 4) and mean rating ± Standard Error for each of the treatments A and B are given in table 1 (b).

Table 1 (b)—*Emerged seedlings of six varieties of barley under ratings 0–4 in treatments A and B on sterilized sand.*

Variety No.	Variety of barley	Treatment	Number of seedlings under ratings 0	1	2	3	4	Total emergence	Mean rating ± S. E.
1.	Spratt archer	A	7	8	5	5	2	27	1.52 ± 0.247
		B	33	0	1	0	3	37	0.38 ± 0.187
2.	Pioneer	A	4	7	5	2	1	19	1.42 ± 0.257
		B	38	0	2	0	1	41	0.20 ± 0.117
3.	Prefect	A	4	4	4	4	1	17	1.65 ± 0.308
		B	31	0	5	2	0	38	0.42 ± 0.149
4.	Plumage archer	A	4	2	4	1	6	17	2.18 ± 0.395
		B	37	0	1	2	1	41	0.29 ± 0.145
5.	Abed maja	A	1	3	4	5	3	16	2.38 ± 0.301
		B	37	0	0	0	2	39	0.21 ± 0.143
6.	Rigel	A	0	1	2	10	1	14	2.79 ± 0.187
		B	31	0	1	0	1	33	0.18 ± 0.134

The mean rating for treatment A is significantly higher than that for B for each of the six varieties of barley. There are no significant varietal differences in the mean ratings for treatment B but in treatment A the varieties Spratt

archer and Pioneer have significantly less mean ratings than Abed maja and Rigel. The detailed results of the tests of significance is summarised with the help of the Bar diagram .

(2) (1) (3) (4) (5) (6)

where the six varieties with their numbers as given in table 1 (b) are arranged according to increasing magnitude of the mean rating, and a single line drawn above or below shows that the varieties which fall within its compass are not significantly different. Thus the above diagram indicates that the mean ratings for varieties (1), (2), (3) and (4) are not significantly different at the 5% level of probability, but the mean ratings for varieties (1) and (2) are significantly less than those for varieties (5) and (6). Similarly the mean ratings for varieties (4) and (5) are not significantly different from that of either variety (3) or (6), but the last two are significantly different.

Pathogenicity tests on three varieties of wheat. The experiment was carried out on similar lines, 50 seeds being used for each of three varieties and for each of the treatments A, B and C. 25 seeds were planted in each pot. The results are given in tables 2 (a) and 2 (b).

Table 2 (a).—*Emergence of three varieties of wheat under treatments A, B and C in sterilized sand.*

Variety of wheat	Treatments			X^2_2	X^2_1		
	A	B	C		C vs. A	C vs B	A vs. B
	Emergence out of 50						
Squareheads Master	29	41	41	9·98**	6.86**	0·00	6·86**
Yeoman	27	39	42	12·50**	10·52**	0.58	6.42*
Holdfast	11	35	39	37·36***	31·36***	0·83	23·19***
X^2_2	15·75***	2.09	0.61				

Table 2 (a) shows that for each variety emergence from seeds infected with *F. avenaceum* is significantly less than that with *F. culmorum* and control seeds, whereas there is no significant difference between the seeds infected with *F. culmorum* and the controls. There are significant varietal differences in the emergence of seeds infected with *F. avenaceum* as can be seen from the values of X^2. The emergence in treatment A is significantly less for Holdfast than for Squareheads Master and Yeoman.

* Significant at 5% level of probability. ** Significant at 1% level of probability. *** Significant at 0·1% level of probability.

Table 2 (b).—*Emerged seedlings of three varieties of wheat under ratings 0-4 in treatments A and B on sterilized sand.*

Variety of wheat	Treatment	Number of seedlings under rating					Total emergence	Mean ratings ± S. E.
		0	1	2	3	4		
Squareheads Master	A	13	6	5	2	3	29	1·17 ± 0·253
	B	39	0	2	0	0	41	0·10 ± 0·068
Yeoman	A	4	5	8	7	3	27	2·00 ± 0·239
	B	32	0	2	3	2	39	0·54 ± 0·194
Holdfast	A	0	3	4	1	3	11	2·36 ± 0·364
	B	33	0	2	0	0	35	0·11 ± 0·080

Table 2 (b) shows that the mean rating for treatment A is significantly higher than for treatment B. There are significant varietal differences in the mean ratings for both treatments A and B. In A Squareheads Master has significantly less mean rating than Yeoman and Holdfast and in B the mean rating for Yeoman is significantly higher than that for Squareheads Master and Holdfast.

Soil microflora and infection. These and later experiments were restricted to *F. avenaceum* and the barley variety Abed maja, which gave higher germination and proved to be more susceptible. Controls were also set up. In this test 100 seedlings were grown in unsterilized and sterilized garden soil taken in pots in each in which 25 seeds were sown. The results are given in tables 3 (a) and 3 (b).

Table 3 (a).—*Emergence of Abed maja under treatments A and C in sterilized and non-sterilized soils.*

Soil Treatment	Treatments		X^2_1
	A	C	
	Emergence out of 100		C vs. A
Sterilized	93	93	0·00
Non-sterilized	90	95	1·80
X^2_1	0·58	0·35	

The four values of X^2 in table 3 (a) indicate that there are no significant differences in the emergence of control and infected seeds in either sterile or non-sterile soil, and none in emergence between sterile and non-sterile soils either in the control or the infected seedlings.

Table 3 (b).—*Emerged seedlings of Abed maja under ratings 0-4 in treatment A in sterilized and non-sterilized soils.*

Soil Treatment	Number of seedlings under ratings					Total emergence	Mean ratings ± S. E.
	0	1	2	3	4		
Sterilized	16	0	14	59	4	93	2·38 ± 0·121
Non-sterilized	57	0	6	23	4	90	1·08 ± 0·154

Table 3 (b) shows that infected seedlings grown in sterile soil have a significantly higher mean rating than those grown in non-sterile soil.

Soil reaction and infection:—Six different levels of pH were used in this experiment by adding suitable amounts of N/10 sulphuric acid or N/30 calcium hydroxide to sterilized soil. The treated soils were dried in air and their pH determined. 20–25 seeds were planted in each pot. 70 seeds were planted for each level at each of the treatments A and C. The results are given in tables 4 (a) and 4 (b).

Table 4 (a).—*Emergence of Abed maja under treatments A and C in sterilized soil at different pH.*

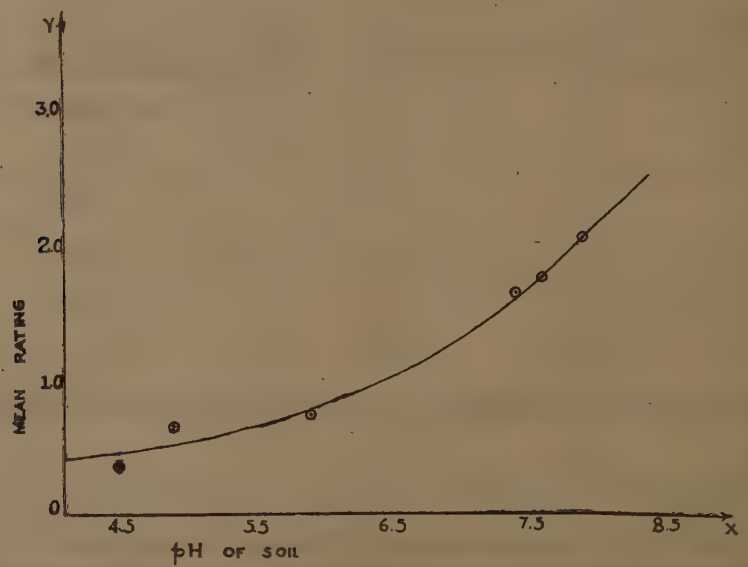
pH of soil	Treatments		X^2_1
	A	C	
	Emergence out of 70		C vs. A
4.5	69	69	0.00
4.9	68	68	0.00
5.9	63	68	2.97
7.4	63	65	0.36
7.6	65	67	0.53
7.9	65	67	0.53
X^2_5	7.48	3.64	

Table 4 (a) shows no significant differences in the emergence of control and infected seeds for each of the six pH levels. Further, the variation in pH has no effect on the emergence of either the control or the infected seeds.

Table 4 (b):—*Emerged seedlings of Abed maja under ratings 0–4 in treatment A in sterilized soils at different pH*

pH of soil	Number of seedlings under ratings					Total emergence	Mean ratings ± S. E.
	0	1	2	3	4		
4.5	60	0	4	4	1	69	0.35 ± 0.113
4.9	52	1	3	10	2	68	0.66 ± 0.152
5.9	46	2	4	7	4	63	0.75 ± 0.168
7.4	24	3	8	26	2	63	1.67 ± 0.180
7.6	24	2	5	32	2	65	1.78 ± 0.180
7.9	18	2	6	37	2	65	2.05 ± 0.169

Table 4 (b) shows that the differences in the mean ratings for the six pH classes are highly significant. There is a tendency for the mean disease rating of the emerged seedlings to increase with the increase of pH. This is shown in Graph I.



GRAPH 1 : Relation between pH of Soil and mean rating.

DISCUSSION

Previous investigators in Britain (Bennett, 1928; Russell, 1931-32; Sadasivan, 1939) have shown *Fusarium culmorum* to be highly pathogenic. My pathogenicity tests on different varieties of wheat and barley in sterile sand under conditions favourable to the activity of the fungi, showed *F. culmorum* to be a weak pathogen and *F. avenaceum* a strong one. All the varieties of barley and wheat tested were attacked by *F. avenaceum* though they showed a difference in susceptibility. In barley, the varieties Spratt archer, Pioneer, Plumage archer, Abed maja and Rigel can be placed in this order of increasing susceptibility to attack by *F. avenaceum*, while in wheat a similar order is Squareheads Master, Yeoman and Holdfast. A test in non-sterilized soil confirmed that *F. avenaceum* is important in causing seedling blight and foot-rot.

Incidence of foot rot due to *F. avenaceum* is insignificant in acid soils, while in alkaline soils, the disease tends to increase with the rise of pH. Control in the field might, therefore, be possible by increasing soil acidity. Barley is an alkaliphilous plant (Small, 1946) and increased acidity is said to reduce the yield of grains. It appears, therefore, that if the soil reaction is kept between pH 6.0-7.0, infection of barley seedlings might be reduced considerably without appreciably affecting yield.

I am grateful to Dr. K. R. Nair, Statistician, Forest Research Institute, Dehra Dun for interpreting the results statistically. I also wish to thank Dr. Malcolm Wilson and Dr. Mary Nobles for their interest in the work, Dr. W. I. Gordon for confirming the identity of the two species of *Fusarium* and Dr. A. M. Smith of the Edinburgh and East of Scotland College of Agriculture, for analysing soil samples.

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KULKARNIELLA, A NEW GENUS OF RUSTS

By V. P. Gokhale and M. K. Patel

(Accepted for publication December 8, 1951)

Leaves of *Pavetta tomentosa* Roxb. (*P. indica* L. var. *tomentosa*) get heavily infected every year by *Aecidium pavettae* Berk. (1, 2 & 3) at Mahableshwar. Attempts have occasionally been made to germinate the aeciospores and to discover the uredial and telial stages but they had not so far met with any success. *Pavetta tomentosa* sheds its leaves in April and May and the new leaves appear from the axillary buds very soon thereafter. These leaves get infected by the rust but the source of the infection had until recently remained, however, a mystery.

An extensive search made during the first week of July, 1951 revealed that the new leaves had become infected but there was no trace of any telia on any of the nearby plants which could have served as the source of infection. A plant of *Pavetta tomentosa* showing marked hypertrophy of the terminal shoot as a result of of previous infection was found in the midst of the infected young leaves. Aeciospores from young and old aecial cups from this plant were collected and attempts were made to germinate them. After about 20 hours, they started germinating but it was observed that germination was by a 4-celled external promycelium, each cell bearing a globular sporidium (Fig. I–B).

It is manifest therefore that the sorus which had been considered to be an aecium was in reality a micro-telium. Young leaves of *Pavetta tomentosa* when inoculated with the aecioid teliospores produced pycnia and fresh aecioid telia after thirty days. Although the infection is observed mostly on the leaves, the apical parts of young shoots when affected by the rust also show marked hypertrophy. The aecioid teliospores germinate readily at temperature ranging from 18° to 21°C. When tested at temperature ranging from 21° to 30°C, only the promycelium was observed.

The method of germination of the spores and the resemblance of the sorus to a true aecium make it nccessary to transfer this rust from the genus *Aecidium* to another genus. Because the pycnia of the rust under study are subcuticular, (Fig. I–A), the rust cannot be placed in the genus *Endophyllum* (4) whose pycnia are subepidermal, deeply seated and with ostiolar paraphyses. It cannot be placed in the genus *Kunkelia* (4) because, though the pycnia are subcuticular, the aecium has a very prominent peridium which is absent in the genus *Kunkelia*. The conspicuous ostiolar paraphyses of the genus *Endophyllum* are also missing in the rust under study.

It has, therefore, become necessary to establish a new genus for the reception of this rust which has the characters, partly of *Endophyllum* (4) and partly of *Kunkelia* (4). It is proposed to establish a new genus and name it *Kulkarniella*, after G. S. Kulkarni, a pioneer mycologist of the Bombay State.

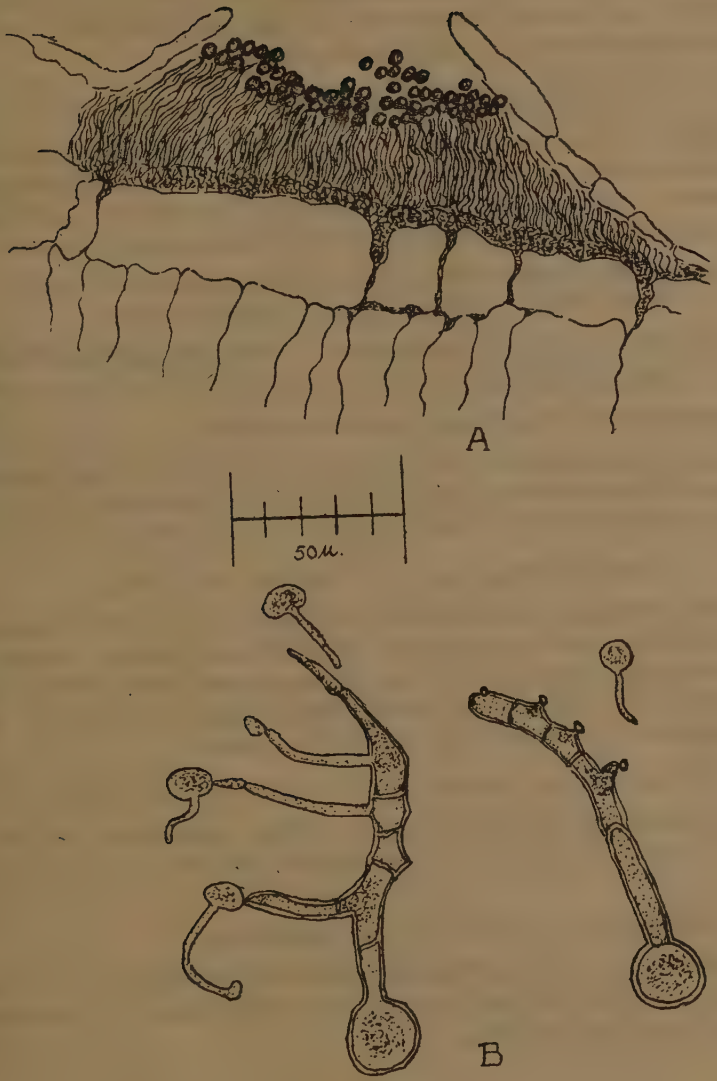

FIG. I.

Dr. Thirumalachar, in a personal letter, states that he has observed normal germ tubes in germination tests with aeciospores of *Aecidium* on *Pavetta tomentosa* occurring in the coffee estates of Mysore and it therefore seems likely that *Kulkarniella* represents a short-cycling state of a long cycled rust. The authors have, however, not observed germination of the rust under study on *Pavetta tomentosa* at Mahableshwar by normal germ tubes.

KULKARNIELLA gen. nov. GOKHALE & PATEL

Pycnia amphigenous, mainly epiphyllous, black, subcuticular, telia amphigenous, mainly hypophyllous, subepidermal, aecioid, with well developed white peridium, erumpent, pulverulent; teliospores cantenulate, easily separable, germinating by a 4-celled external promycelium bearing globular sporidia.

Type species :—*Kulkarniella pavettae* on *Pavetta tomentosa* Roxb.

KULKANIELLA PAVETTAE sp. nov. GOKHALE & PATEL

Characteristics same as those of genus. Pycnia amphigenous 150–190 μ in section. Teliospores globoid or more or less angular, 20–25 x 17–21 μ, yellow.

On leaves of Pavetta Tomentosa at Mahalbeshwar.

Type specimens deposited in the herbaria of the Plant Pathologist to the Government of Bombay, Poona, the Indian Agricultural Research Institute, New Delhi and Commonwealth Mycological Institute, Kew, England.

KULKARNIELLA gen. nov. GOKHALE AND PATEL

Pycnia amphigena, sed ut plurimum epiphylla, nigra, subcuticularia; telia amphigena, sed ut plurimum hypophylla, subepidermalia, aecioidea, peridio bene evoluto ornata, erumpentia, pulverulenta; teliosporae catenulatae, faciliter separabiles, unicellulatae, germinantes per promycelium externum 4-cellulatum. Species typica : Kulkarniella pavettae in foliis *Pavettae tomentosae*.

KULKARNIELLA PAVETTAE sp. nov. GOKHALE AND PATEL

Notae characteristicae ut in genere. Pycnia amphigena, 150–190 μ in sectione. Teliosporae globoidea vel plus minusve angularia, 20–25 x 17–21 μ luteae.

In foliis Pavattae Tomentosae in loco Mahableshwar.

Typus positus in herbario Plant Pathologist to the Government, Bombay, in civitate Poona; in Ind. Agric. Res. Institute, New Delhi, atque in Commonwealth Mycological Instit., Kew in Anglia.

SUMMARY

The *Aecidium* on *Pavetta tomentosa* at Mahableshwar has been found to be not an Aecidium at all, but a microcyclic rust. The spores so long considered to be aeciospores have, on germination, been found to be micro-teliospores, as they germinate by the formation of a 4-celled promycelium with a single sporidium in each cell. The rust cannot be accommodated in the genus *Endophyllum* as it has

subcuticular pycnia and does not have ostiolar paraphyses. It cannot be placed in the genus *Kunkelia* as the aecium has a very prominent peridium which is absent in the species of that genus. A new genus for the reception of this rust has been established and named *Kulkarniella*.

The authors are sincerely grateful to Drs. B. B. Mundkur and M. J. Thirumalachar for help rendered in the preparation of this article. Thanks are also due to Rev. Fr. H. Santapau for Latin diagnosis of the genus and species.

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ADAPTATION OF FUNGI TO FUNGICIDES AND ITS SIGNIFICANCE IN AGRICULTURE

By M. L. Gattani

(Accepted for publication December 7, 1951.)

One of the important questions during recent years in Plant Pathology has been whether fungi can be educated to adapt themselves to increasing concentrations of toxic substances. The Minnesota group of investigators under Stakman have made some significant contributions to the subject. Stakman, Stevenson and Wilson (1946) stated that haploid lines of maize smut showed a definite increase in tolerance to arsenic in the media, if they were grown on a series of ever increasing concentration of sodium arsenite. This tolerance or ability to grow in the presence of arsenic could be raised to a considerable degree over that of any line not having such treatment. Gattani (1946) showed that as a result of successive transfer from lower to higher concentration of sodium arsenite, diploid lines of maize smut like their haploid parents, developed the ability to grow on media containing approximately six times the concentration of sodium arsenite as the lines would tolerate initially. Christensen (1946) showed that monoconidial isolate of *Giberella zeae* developed increased tolerance to malachite green, mercuric chloride and ethyl mercuric phosphate as a result of successive transfer from media containing lower concentrations of these substances to media containing higher concentrations. Hirschhorn and Munnecke (1950) in an attempt to evaluate whether the increase in tolerance of the haploid lines 10A4 and 17D4 of maize smut to arsenic was due to mutation, made crosses between the adapted lines to see if the increased tolerance could persist through sexual generation. They found that one of the adapted lines lost its ability to combine with the line of opposite sex and did not produce galls and chlamydospores in inoculated maize plants. This was not true, however, of the other line. From whatever has been stated it is clear that fungi do educate themselves to increasing concentrations of toxic substances although no categorical statement can be made whether the adaptation is due to mutation or not. These findings raise an important question. If fungi could adapt themselves to increasing concentrations of toxic substances, all our attempts to control a plant disease by a particular fungicide are likely to defeat their own purpose. The present work was undertaken with a view to find out a possible solution to this problem.

MATERIALS AND METHOD

The fungus used in these studies was a species of *Alternaria* isolated from wheat seeds. The *Alternaria* sp. was found to bring about a rot of wheat seedlings. Monoconidial isolations were made by the dilution method and culture of the fungus was established. The fungicides used in these investigations were Agrosan GN (fungicide with an organic mercuric compound as the active ingredient) and Arasan (50 percent tetra-methyl-thiuram-di-sulfide). To prepare media containing 50, 100, 200, 300, 500, 700 etc. units of fungicide in million units of potato

dextrose agar, 0.05, 0.1, 0.2, 0.3, 0.5 and 0.7 gm. of fungicide were incorporated into 1,000 gm. of sterile potato dextrose agar while it was still hot. The agar after incorporation of the fungicide was thoroughly shaken so as to give the fungicidal dust a uniform dispersion in the medium. Twenty five ml. portions of the agar medium containing different dilutions of the fungicide were poured out into sterile petri plates and the plates inoculated after the agar had set. A sharp edged sterile cork borer was employed to cut equal and standard size of inoculum in each case. All the plates were incubated at 28°C. Final readings of the colony size were taken after fifteen days of growth.

EXPERIMENTAL RESULTS

In the first series of the experiment inoculum from the monoconidial isolate of *Alternaria* sp. from potato dextrose agar was grown on potato dextrose agar and potato dextrose agar containing 100, 200, 300, 500, 700 and 1,000 p.p.m. of Agrosan GN. The maximum colony size attained on all these media after 15 days is shown in Table 1. On potato dextrose agar containing upto 300 p.p.m. of Agrosan GN there was a gradual decrease in colony size from lower to higher concentration of the fungicide. No growth of the fungus occured on media containing 500, 700 and 1,000 p.p.m. of the fungicide even after thirty days thus indicating that these doses of the fungicide were toxic to the fungus.

In the second series of the experiment the inoculum used was from the fungal colony grown on 1000 p.p.m. of the fungicide in the experiment of the first series. It was transferred to media containing 100, 200, 300, 700 and 1,000 p.p.m. of the fungicide. It would appear from Table 1 that the fungus which had been grown for one generation on medium containing 100 p.p.m. of the fungicide showed better growth on media containing 100, 200 and 300 p.p.m. of the fungicide, and it also grew on medium containing 500 p.p.m. of the fungicide.

In the third series of the experiment the inoculum used was from the fungal colony grown on medium containing 300 p.p.m. of the fungicide of the second series. It was transferred to the different media of the Agrosan GN series. As would be seen from Table 1 in this case the fungus grew better on all the concentrations of the fungicide than in the first and second series. On medium containing 500 p.p.m. of the fungicide, colony size was almost double than that in the second series. In the fourth series of the experiment the inoculum used was from medium containing 300 p.p.m. of the fungicide of the third series. It had thus grown for three successive generations on Agrosan GN media. As would appear from Table 1, in this series of the experiment the fungus showed still better growth on media containing upto 500 p.p.m. of the fungicide. Not only that, in this series it also grew on medium containing 700 p.p.m. of the fungicide. The colony of the fungus on this concentration of the fungicide was, however, concave and bushy. It would be seen from this experiment that the adapted line showed better growth than the unadapted line when both were transferred to media of the Agrosan GN series.

Table 1—*Size in mm. of the colonies of Alternaria sp. after 15 days on media containing increasing concentrations of Agrosan GN when the fungus was transferred directly from potato dextrose agar and after being grown for one, two and three generations on media containing Agrosan GN.*

Series	Source of Inoculum	Replications	Colony size in mm.						
			PDA	Concentration of Agrosan GN in p.p.m.					
				100	200	300	500	700	1000
1	potato dextrore agar	1	79	60	55	45	x	x	x
		2	80	58	56	44	x	x	x
		3	80	60	55	43	x	x	x
2	100 p. p. m. medium of first series	1	80	72	60	48	3	x	x
		2	79	70	64	49	5	x	x
		3	80	70	62	50	6	x	x
3	200 p. p. m. medium of second series	1	80	75	69	50	10	x	x
		2	79	74	69	52	11	x	x
		3	80	73	72	54	14	x	x
4	300 p. p. m. medium of third series	1	80	74	68	56	25	4	x
		2	79	75	70	54	24	5	x
		3	80	73	72	53	27	4	x

In the second experiment potato dextrose agar media containing 50, 100, 500, 700, 1000, 1500, and 2000 p. p. m. of Arasan were prepared. The fungus used as inoculum in the first series of this experiment was from potato dextrose agar; in the second series from medium containing 100 p. p. m. of Arasan of the first series; in the third series from medium containing 500 p. p. m. of Arasan of the second series and in the fourth series from medium containing 700 p. p. m. of Arasan of the third series. It would appear from table 2 that when the fungus is transferred directly from potato dextrose agar to media containing increasing concentration of Arasan, it does not grow on media containing 1000, 1500 and 2000 p. p. m. of Arasan. When the fungus had been grown for one generation on the medium containing 100 p. p. m. of Arasan and then transferred to the Arasan series media, it showed better growth than when it was transferred directly from potato dextriose agar to the Arasan series. In the former case it grew on medium

containning 1000 p. p. m. of Arasan whereas in the latter case it did not grow on this concentration of Asaran. The fungus which had been grown for two generations on Arasan thrived even better than the fungus which had only been grown for one generation, as it could tolerate a concentration of 1500 p. p. m. of the fungicide. In the fourth series of the experiment the inoculum used was from the fungus which had been grown for three generations on Arasan media. It thrived still better, the colony size being bigger on media containing different concentrations of Arasan. It also grew on medium containing 2000 p. p. m. of Arasan. It, therefore, appears that the unadapted line of *Alternaria* sp. could only tolerate a concentration of 700 p. p. m. of the fungicide in potato dextrose agar but the adapted line (line which had been grown for three generations on media containing Arasan) could tolerate a concentration of 2000 p. p. m. of Arasan in potato dextrose agar.

Table 2—*Size in mm. of the colonies of Alternaria sp. after 15 days on media containing increasing concentrations of Arasan when the fungus was transferred directly from potato dextrose agar and after being grown for one, two and three generations on media containing Arasan.*

Series	Source of Inoculum	Replications	Colony size in mm.							
			PDA	Concentration of Arasan in p. p. m.						
				50	100	500	700	1000	1500	2000
1	Potato dextrose agar	1	80	68	45	16	6	x	x	x
		2	79	70	48	18	8	x	x	x
		3	80	68	47	17	7	x	x	x
2	100 p. p. m. medium of first Series	1	80	72	50	35	19	8	x	x
		2	79	74	52	33	18	7	x	x
		3	80	72	53	35	17	8	x	x
3	500 p. p. m. medium of second Series	1	80	72	55	40	22	15	5	x
		2	80	73	54	41	23	17	5	x
		3	79	74	55	42	24	16	6	x
4	700 p. p. m. medium of third Series	1	80	76	60	48	39	20	8	x
		2	80	77	61	47	40	23	7	3
		3	80	76	60	46	41	22	8	2

In the next experiment the line of *Alternaria* sp. adapted to Agrosan GN by growing it on Agrosan GN media for three generations and the unadapted line were transferred to media containing increasing concentrations of Arasan. The inoculum used for the adapted line was from the flask containing 300 p. p. m. of Agrosan GN in potato dextrose agar in the third series of Table 1. On being transferred to the Arasan series, the line which had been adapted to Agrosan GN behaved like the unadapted line i. e. the line which was directly transferred from potato dextrose agar to the Arasan series as illustrated in Table 3. This means that the line was only adapted in respect to Agrosan GN but behaved as an unadapted line in respect to Arasan.

Table 3—*Behaviour of the unadapted line and the line adapted on Agrosan GN on Alternaria sp. when grown on potato dextrose agar containing increasing concentration of Arasan.*

Source of Inoculum	Replications	Colonysize in mm.							
		PDA	Concentration of Arasan in p. p. m.						
			50	100	500	700	1000	1500	2000
Potato	1	80	67	45	16	8	x	x	x
dextrose	2	79	68	47	18	9	x	x	x
agar	3	80	69	48	17	8	x	x	x
300 p. p. m.	1	80	65	46	18	6	x	x	x
medium of third	2	79	67	46	17	7	x	x	x
series Table 1	3	80	66	45	18	8	x	x	x

In the next experiment the unadapted line of *Alternaria* sp. from potato dextrose agar and the line of *Alternaria* sp. adapted on Arasan by growing it on Arasan media for three generations was transferred to the media of Agrosan GN series. The results are recorded in Table 4. It would appear that the line adapted on Arasan when transferred to media containing different concentrations of Agrosan GN behaved like the unadapted line. Both the lines did not grow on media containing 500 p. p. m. of Agrosan GN.

Table 4—*Behaviour of the unadapted line and the line adapted on Arasan of Alternaria sp. when grown on potato dextrose agar containing increasing concentrations of Agrosan GN.*

Source of Inoculum	Replications	Colony size in mm. Concentration of Agrosan GN in p.p.m.						
		PDA	100	200	300	500	700	1000
Potato	1	80	60	56	45	x	x	x
dextrose	2	79	59	54	44	x	x	x
agar	3	80	60	56	43	x	x	x
500 p.p.m.	1	80	60	56	43	x	x	x
Medium of	2	79	60	55	42	x	x	x
third Series Table 2	3	80	61	55	41	x	x	x

DISCUSSION

The investigations of Stakman et al (1946), Gattani (1946), Christensen (1946), Hirschhorn and Munnecke (1950) have shown that fungi can adapt themselves to increasing amounts of toxic substances, as a result of successive transfer from media containing lower to higher concentrations of toxic substances. The author has found that *Alternaria* sp. studied by him also got adapted to increasing concentrations of Agrosan GN and Arasan as a result of successive transfers from media containing increasing amounts of these substances. The adaptability of the fungus was directly correlated with the number of generations for which the fungus had been grown on the media containing Agrosan GN or Arasan. For example the line which had been grown for three generations on media containing Agrosan GN or Arasan showed better growth on media containing increasing amount of the fungicides than the line which had been grown for one generation only.

The most significant fact of this investigation is that when the line adapted on Agrosan GN was transferred to the Arasan series, it behaved as an unadapted line. The same was true of the line which had been adapted on Arasan. On transfer to Agrosan GN series it behaved as an unadapted line. Rammelkemp and Maxon (1942) got somewhat similar results in their studies on resistance of *Staphylococcus aureus* to the action of penicillin. They found that the development of resistance to penicillin does not affect the response of this organism to the therapeutic action of the sulfonamide drugs nor development of resistance to sulfonamides appear to interfere with the antibacterial activity of penicillin.

The fact that the line adapted on Agrosan GN behaves as an unadapted line on being transferred to the Arasan series and *vice versa* provides a clue to the problem of adaptation of fungi in relation to the control of diseases with fungicides. When plants are sprayed or dusted with fungicides it is possible that the fungus as a result of exposure to the fungicide for a considerable period, may adapt itself, to that particular fungicide. Continuous use of that fungicide may, therefore, prove ineffective for the control of that disease. In light of these investigations in such a case to achieve effective control of the plant disease the obvious remedy would be to alternate the type of fungicide. It would be necessary to use only those fungicides for this purpose which have different chemical constituents as their active ingredients.

The author is grateful to Prof. E. C. Stakman of the University of Minnesota, U. S. A. for many helpful suggestions.

SUMMARY

Alternaria sp. isolated from wheat seeds got adapted to increasing concentration of Agrosan GN and Arasan as a result of successive transfers from media containing increasing amounts of these substances. However the line adapted on Agrosan GN when transferred to the Arasan series behaved as an unadapted line. Similarly the line adapted on Arasan behaved as an unadapted line on transfer to the Agrosan GN series. The author in view of these investigations suggests the practice of alternating the type of fungicide for the control of a plant disease in case continuous use of fungicide becomes ineffective.

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FUSARIUM WILT OF LANG (LATHYRUS SATIVUS L.): ISOLATION OF WILT RESISTANT TYPES

By V. P. Bhide, M. K. Patel, and G. W. Dhande

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Lang (*Lathyrus sativus* L.) wilt caused by *Fusarium orthoceras* var. *lathyri* Bhide and Uppal has assumed serious proportions in the Broach district of Bombay State. Since *Fusarium* wilts are not amenable to direct control, selections of wilt resistant varieties is the only method of combating these diseases. The following pages give an account of the work done in developing varieties of *lang* resistant to wilt.

MATERIALS AND METHODS

Bhide and Uppal (1948) have demonstrated that soil-temperature is a limiting factor in the development of wilt in *lang*. According to Uppal (1939) the isolation and fixation of strains resistant to wilt should be done under optimum conditions for infection and he has also described the technique involved in producing wilt resistant strains of cotton. This technique was first developed at Poona for the isolation of wilt resistant varieties of *sann-hemp* (*Crotalaria juncea* L.) and the same procedure was followed in the present work.

The material used was supplied by the Director, Institute of Plant Industry, Indore. In all, four varieties of *lang* were obtained. Selections were also made by the senior author in the Broach district in 1938 and 1939, and at Poona in an artificially wilt infested plot.

FIELD TESTS

Giant cultures of the fungus were grown in solution and they were mixed with field soil and farm-yard manure. When the fungus had grown sufficiently, this mixture was spread evenly in the plot which was about 1/20th acre in area, and mixed thoroughly by harrowing. A crop of *lang* locally grown at Broach was raised in this plot. When the plants had started flowering, the crop was uprooted, cut into pieces and mixed with wilt infested soil. This mixture was then 'composted' in a pit for one season and then spread in the plot. Another crop of local *lang* was grown in the plot and 'green-manured' when flowering had set in. The field tests were started in this plot at the College of Agriculture, Poona, in the cold season of 1938.

Season 1938-1939. In October, 1938, seeds of four types of *lang* were received from Indore as previously stated. These were, viz., Type 1- plant 2; Type 1- plant 37; Type 6- plant 3; and Type 148- plant 2. These types were sown in the wilt infested plot in late October 1938; a few lines of Broach local *lang* were sown as control.

The authors are indebted to the Sir Sasoon David Trust Fund for financial help in this investigation and also to Dr. B N. Uppal, Director of Agriculture, B. S. Poona for advice and guidance.

As the plot had not become heavily infested with the wilt organism the disease did not appear to a very great extent; Broach local *lang* sufferred a mortality of about 40 per cent; Type 1– plant 37 and Type 148–plant 2 remained free from wilt but the others segregated and showed varying degrees of wilt infection.

As *lang* is a self-fertilised plant, no difficulty was experienced in obtaining selfed seed by bagging the selected plants individually. When the plants had dried at the end of the season, the pods from each plant were carefully harvested and the seeds were stored in stopperred glass vials till the next season to prevent infestation by 'pulse beetle' to which *lang* is very susceptible. Table 1 summarises the results of this test.

Table 1—*Wilt reactions of lang types in the infested plot at Poona, 1938–39.*

Type of *lang*	No. of effective plants	Per cent wilt	No. of plants selected
Type 1, plant 2	143	11.2	17
Type 1, plant 37	18	0.0	18
Type 6, plant 3	107	18.7	21
Type 148, plant 2	18	0.0	18

Season 1939–1940. The selfed seeds of selected plants from the previous season's trial were resown in the infested plot in October 1939. The original names of the types were replaced by new ones to insure brevity and a uniform system of nomenclature. This was done as follows:

OLD NAME	NEW NAME
Type 1, plant 2	Indore T. 1
Type 1, plant 37	Indore T. 2
Type 6, plant 3	Indore T. 3
Type 148, plant 2	Indore T. 4

Seeds of selections made in the infested plot at Poona in the previous season were also sown along with the selected material from the above types. Large quantities of wilt compost were spread in the plot before sowing. Wilt appeared about a month after sowing of seeds. Progenies of the Indore types segregated and showed wilt infection varying from 0 to 22.8 per cent in Indore T.1, 0 to 45 per cent in Indore T. 2, 0 to 63.3 per cent in Indore T. 3, and 0 to 6. 6 per cent in Indore T. 4. The Poona selections showed wilt incidence varying between 0 and 100.0 per cent. When flowering had started, selections were again made from amongst the survivals and these were bagged to obtain selfed seed. After harvest of pods, each bagged plant was carefully uprooted and the root system examined

for vascular discolouration. Selfed seeds of only such plants that were free from root discolouration were preserved for further testing. Table 2 summarises the results of this trial.

Table 2—*Wilt reactions of selections in field test, 1939-40.*

Type	No. of progenies tested	Total No. of plants tested	Intensity of wilt infection per cent	No. of selections made
Indore T. 1	17	834	0.0- 22.8	11
Indore T. 2	18	725	0.0- 45.0	41
Indore T. 3	21	640	0.0- 63.3	17
Indore T. 4	18	210	0.0- 6.6	10
Poona S. 1	14	179	0.0-100.0	1

It will be seen from the results recorded in Table 2 that quite a few progenies from each type (except Poona S. 1) remained completely free from wilt under field conditions of infection. It was therefore decided to test this promising material in pot culture in a glasshouse under controlled conditions for wilt infection. The remaining material which had not yet reached homozygosity for resistance to wilt under field conditions of testing was further tested in the infected plot for two more seasons. None of the selections showed promise of yielding resistant types and this material was eventually discarded.

GLASSHOUSE TESTS

Season 1940-1941. It has been previously stated that some progenies from the Indore types and one selection from Broach local *lang* (grown at Poona and named Poona S. 1) did not suffer any mortality from wilt in the field test carried out during the 1939-40 season. This material was tested in pot culture under controlled conditions for wilt infection in a glasshouse at Poona.

The wilt fungus was grown on Richards' solution and infested soil was prepared by mixing the fungus growth with steamed red (river bottom) soil. The fungus growth from one Erlenmeyer flask containing 250 ml. Richards' solution was mixed with enough soil to fill four earthen pots of four inch size. This quantity of inoculum was maintained throughout the succeeding tests. When the fungus had properly grown in the soil, pots–previously disinfected by soaking in a five per cent solution of copper sulphate— were filled and kept on the glasshouse bench. Four seeds were sown in each pot; Broach local *lang* served as control. An attempt was made to maintain the soil temperature between 25° and 28° C. The soil and air temperatures in the glasshouse were recorded four times daily, the minimum soil temprature recorded during the test was 24°C. and the maximum was 30°C. The pots were watered whenever necessary and the surface soil stirred occasionally. Sowing

was done on October 28, 1940. Wilt appeared eight days after emergence of seedlings and progressed rapidly thereafter. Progenies of cultures Indore T. 2–1 and Indore T. 3–8 only remained completely free from wilt whilst the rest segregated and showed varying amounts of infection. Broach local *lang* (control) showed about 80 per cent mortality. Table 3 summarises the results of this test.

Table 3—*Wilt reactions of lang selections in pot culture tests in a glasshouse at Poona, 1940–41.*

Culture	No. of progenies	Total No. of plants tested	Intensity of wilt infection per cent	No. of selections made
Indore T. 1–17	2	94	0.0–54.1	12
Indore T. 2–1	1	44	0.0	13
Indore T. 2–4	1	22	40.9	0
Indore T. 2–10	4	232	7.3–28.6	0
Indore T. 2–11	4	265	10.6–12.0	0
Indore T. 2–12	5	118	0.0–13.6	20
Indore T. 2–13	2	82	0.0– 4.4	21
Indore T. 2–14	2	58	22.2–22.5	0
Indore T. 3– 1	2	60	0.0–18.8	17
Indore T. 3– 7	1	33	9.0	8
Indore T. 3– 8	1	29	0.0	9
Indore T. 3– 9	2	58	7.6–33.3	0
Indore T. 4	11	399	23.1–90.5	35
Poona S. 1	1	88	13.6	16

When wilting had ceased, selections were made from amongst the survivals and transplanted in the wilt infested plot. A large number of the transplants died in the field as a result of high air temperature and other unfavourable conditions. Some died of wilt. When flowering had set in, every plant was enclosed in a muslin bag in order to obtain selfed seed. When the plants had almost dried up, the seeds from each plant were carefully harvested, the plants were uprooted and the roots examined for vascular discolouration. Selfed seeds of plants, free of vascular discolouration, were preserved for further tests.

Seasons 1941-1942 and 1942-1943. Inbred progenies of selections which had not suffered any mortality from wilt in the preceding season were further tested in pot culture in a glasshouse during the 1941-42 and 1942-43 seasons. Progenies of all cultures except those of Indore T. 2-1 segregated and showed varying amounts of infection from wilt. Table 4 summarises the results of these two tests.

Table 4.—*Wilt reactions of lang selections in pot culture tests in a glasshouse at Poona, 1941-42 and 1942-43.*

Culture	No. of progenies and total no. of plants tested		Intensity of wilt infection, per cent	
	1941-42	1942-43	1941-42	1942-43
Indore T. 1–17	4/121*	5/392	6·4-13·3	17·1-31·3
Indore T. 2–1	4/41	9/441	0·0	0·0
Indore T. 2–12	13/276	15/694	0·0-23·5	0·0-37·4
Indore T. 2–13	4/98	discarded	5·6-25·0	discarded
Indore T. 3-1	2/23	,,	25·0-33·3	,,
Indore T. 3–7	4/73	2/66	5·5-35·7	3·2-20·0
Indore T. 3–8	5/106	1/40	0·0-18·2	0·0
Indore T. 4–5	6/113	4/46	0·0-30·0	0·0-26·9
Indore T. 4–9	5/99	discarded	5·0-75·0	discarded
Indore T. 4–10	6/144	1/14	0·0-25·0	7·1
Indore T. 4–11	8/107	5/201	0·0-5·00	6·6-92·8
Poona S. 1	2/67	7/220	0·0-3·3	0·0-7·4

* Numerator denotes the number of progenies and the denominator denotes the total number of plants.

Since the culture Indore T. 2–1 had shown complete resistance to wilt in five successive tests— two in the field and three in the glasshouse in pot culture tests— it was presumed that it had reached homozygosity for resistance to wilt and further testing of this culture was stopped. Selfed seeds of this culture were sown in a small plot at Poona for seed multiplication and the resulting mass seed was utilised for village trials in the Broach district.

Of the remaining cultures, only two, viz., Indore T. 2–12 and Poona S. 1 were selected for further testing as they showed promise of yielding wilt resistant types; the rest of the cultures were discarded.

The above two cultures were tested in pot culture in the glasshouse at Poona for five more seasons in the usual manner. At the end of this period, culture Indore T. 2–12 had reached complete homozygosity for resistance to wilt whilst culture Poona S. 1 was still segrerating. Further work was stopped in 1948–49 when the project was teminated. Table 5 shows the condensed results of these five successive tests.

Table 5—*Performance of cultures Indore T. 2–12 and Poona S. 1 in pot culture tests in a glasshouse at Poona 1943–1948.*

Season	Indore T. 2–12		Poona S. 1	
	No. of progenies	Intensity of wilt infection per cent	No. of progenies	Intensity of wilt infection per cent
1943–44	15	0.0–5.5	12	0.0
1944–45	16	0.0–9.3	39	0.0–50.0
1945–46	13	0.0–1.6	61	0.0–25.0
1946–47	12	0.0	60	0.0–21.7
1947–48	11	0.0	51	0.0–9.1

VILLAGE TRIALS OF THE RESISTANT STRAINS

Since the cultures Indore T. 2–1 and Indore T. 2–12 had reached complete homozygosity for resistance to wilt, it was decided to undertake village trials with these strains as a preliminary to advocating their general cultivation in the Broach district. Along with these two strains, Poona S. 1 was also used in the trials (even though it was not completely wilt resistant) as it had been evolved from Broach local *lang*.

These trials were repeated for two successive seasons, 1946–47 and 1947–48; the lay-out was a randomised block arrangement of six replications of three wilt resistant strains of *lang* with Broach local *lang* as control. The net size of each plot was 1/40 acre. During the first season, the trials were conducted only at one place–Haldhar near Broach–but during 1947–48, the trials were carried out at Haldhar, Bori, and Kuwadar. The lay-out and conduct of the experiments was essentially the same each year. These trials gave an indication of the yielding capacities of these improved strains in addition to proving their wilt resistant character under field conditions of growth. Table 6 summarises the results of these trials.

Table 6—*Results of village trials of wilt resistant lang strains, 1946--47 and 1947--48*

Strain	1946-47		1947-48	
	Wilt* Per cent	Yield* lb.	Wilt Per cent	Yield lb.
Indore T.2-1	0.0	17.6	0.0	4.1
Indore T.2-12	0.1	20.0	0.0	4.5
Poona S.1	not tested	not tested	8.3	2.3
Control (local)	25.0	14.2	26.0	2.1

* These are mean figures. Yield figures are per 1/40th of an acre.

It was observed during these trials that although the two strains Indore T. 2--1 and Indore T. 2--12 were resistant to wilt, there were some deaths due to *Macrophomina phaseoli*. The general yields in 1947--48 were very low on account of unfavourable season. The above results on yield were analysed statistically and it was found that the yields of the two Indore strains were significantly higher than those of Poona S. 1 and Broach local *lang*.

The wilt resistant strains Indore T. 2-1 and Indore T. 2-12 are now being multiplied on a large scale with a view to distributing their seeds for general cultivation in the Broach area.

SUMMARY

Wilt of *lang* (*Lathyrus sativus* L.) is a limiting factor for the successful cultivation of *lang* in the Broach area, and it was therefore decided to isolate wilt resistant strains of this crop for replacing the existing wilt susceptible variety.

Four types of *lang* were received from the Director, Institute of Plant Industry, Indore, and these in addition to selections made in the Broach district and at Poona, constituted the material for the tests.

This material was tested under field conditions of infection in an artificially wilt infested plot at Poona for two successive seasons in 1938-39 and 1939-40, and plants showing a high degree of resistance to wilt were selected for further trials.

Progenies of cultures that did not suffer any mortality from wilt under field conditions of infection were further tested in pot culture in a glasshouse under optimum conditions for infection. Selections were made from amongst the survivals and these were transplanted in a wilt infested plot. Selfed seed obtained from these transplants was further tested in the glasshouse in the next season.

Culture Indore T. 2–1 remained completely free from wilt since the beginning of the trials and did not segregate throughout the successive tests in the glass-house. The rest of the cultures when further tested in a similar manner yielded another wilt resistant strain, namely, Indore T.2–12.

These two strains were tested in the field at various places in the Broach area and proved to be superior to the local *lang* in their yields in addition to their being completely wilt resistant.

These two strains are now advocated for general cultivation in the Broach district.

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Poona 5.

REFERENCES

Bhide, V. P. and Uppal, B. N. (1948) A new *Fusarium* disease of *lang* (*Lathyrus sativus*). *Phytopathology* **33**: 560-567.

Uppal, B. N. (1939) Breeding for Wilt Resistance in Cotton. *Conf. Sci. Res. Workers on Cotton, India. Cotton Mycology Paper* No. 1.

FOURTH ANNUAL REPORT OF THE INDIAN PHYTOPATHOLOGICAL SOCIETY (1950)

I am submitting herewith the Fourth Annual Report for 1950 of the Indian Phytopathological Society. At the end of 1949 the total membership was stated to be 196, of whom 28 had not paid their 1949 dues. In spite of several reminders 22 members did not pay their subscription and they may be considered as having resigned the membership of the Society. During the year under report 21 new members were enrolled, of whom one was a Life Member. The total number of members in good standing now is 174; one is a Patron, 39 are Life-Members of whom 5 are paying their dues in instalments, and 134 members are paying their subscriptions annually.

Volume II, Nos. 1 and 2 of Indian Phytopathology were published during this year. The Journal has proved very popular in foreign countries, specially the U. S. A., thanks to the efforts of Dr. B. B. Mundkur, my predecessor in office, and we have now 82 libraries in India and abroad on our list of subscribers but out of these only 27 are Indian. Efforts should, therefore, be made by individual members to induce more libraries and institutions in this country to subscribe to our Journal.

On account of difficulties with the British India Press, the work of printing the Journal had to be entrusted to another press. This has meant so much delay in bringing out Vol. III. In spite of our best efforts, it has not been possible to issue the 1950 volume so far. The first number is, however, in the galley proof stage. Since the work has now been entrusted to a local press in Delhi, it would be possible to expedite the printing of the journal and we hope to issue both the numbers of Vol. III by the end of June at the latest.

Putting aside a sum of Rs. 5,000 invested in National Savings Certificates, the year began with Rs. 4,762-8-7 to our credit. Receipts during the year amounted to Rs. 6,351-8-3 which includes Rs. 200 given by the National Institute of Sciences of India as donation and Rs. 1,268-8-0 granted by the Indian Council of Agricultural Research as subsidy towards the printing of two numbers of Indian Phytopathology during 1950-51. The efforts of my worthy predecessor have thus borne fruit. The expenses incurred have amounted to Rs. 6,588-14-9. This is due to the fact that payment was made this year for printing *three* issues of the journal, namely, Vol. I, No. 2 and Vol. II, Nos. 1 and 2, in place of two issues per year and also on account of increase in the printing rates and cost of paper. A second hand type writer was purchased for Rs. 100. The accounts for 1949 were audited by a Chartered Accountant at a cost of Rs. 25 and are placed before you. They will be published in the next issue of the journal. A tentative statement of Receipts and Expenditure for 1950 has been prepared and will be properly audited by a Chartered Accountant as in the previous years.

On account of rise in the price of paper and of printing charges, the cost of issuing two numbers of the Journal per year would amount to not less than

Rs. 3,500. It is obvious that it will not be possible to meet this expenditure unless there is a larger membership and an increase in the number of subscribers. As pointed out in the last Annual Report, many Institutes, Universities and Colleges in India have not yet subscribed to the Journal. Unless we can increase the income of the Society by securing more members and subscribers, either the annual subscription will have to be increased or the standard of the Journal lowered. I am sure the members would not like to economise in the latter direction. It is, therefore, before you to consider the ways and means to increase the revenues of the Society. In my opinion the membership fee will have to be raised to Rs. 12/8 per annum and the subscription of the Journal to Rs. 18 or £1-6-0 foreign per volume. Unless you agree to this, I am afraid, it would not be possible to make the two ends meet, in spite of all economies.

Our grateful thanks are due to the Indian Council of Agricultural Research for subsidising the printing of the Journal by 33 per cent and to the National Institute of Sciences of India for the grant of Rs. 200.

I must express my grateful thanks to the members of the Society for their kind co-operation, and to the Members of the Council in general and Dr. B. B. Mundkur, President of the Society and Dr. R. S. Vasudeva, the Editor-in-Chief, in particular, for the support, encouragement and advice which I have received from them.

R. PRASAD

28–12–50 *Secretary-Treasurer.*

THE INDIAN PHYTOPATHOLOGICAL SOCIETY, DELHI

Receipts and Payments account for the year ended 31st December, 1950.

Receipts							Payments						
To Opening Balance :—							By Printing and Stationery				6,057	4	0
Cash in hand	72	7	0				,, Postage				116	1	6
Balance with Lloyds Bank Ltd., New Delhi in Savings Account	4,690	1	7	4,762	8	7	,, Audit Fees				25	0	0
							,, Cost of Typewriter				100	0	0
To Admission Fees				180	0	0	,, Travelling Expenses of Secretary				115	0	0
,, Life Membership Fees				305	0	0	,, Bank Charges				52	12	0
,, Subscriptions :—							,, Conveyance				18	8	6
For 1948	58	0	0				,, Miscellaneous Expenses				104	4	9
,, 1949	164	4	0				,, Closing Balance :—						
,, 1950	1,353	7	0				Cash in hand	185	8	3			
,, 1951	103	0	0				Balance with Lloyds Bank Ltd., New Delhi, in Savings Account	4,338	9	10	4,524	2	1
,, 1952	10	0	0	1,688	11	0							
,, Subscriptions for journal				2,015	1	3							
,, Reprints				609	9	0							
,, Advertisements in journal				36	0	0							
,, Interest on Savings Account				37	11	0							
,, Publication grants				1,468	8	0							
,, Amount refundable				10	0	0							
				11,113	0	10					11,113	0	10

We have Examined the above Receipts and Payments Account and Certify that it has been found correct in accordance with the books and information supplied.

We have also verified that Post Office 12 year National Savings Certificates of the value of Rs. 5,000 belonging to the society was held in safe custody with Lloyds Bank Ltd., as on 31-12-50

New Delhi
Dated 14-7-51

R. Prasad.
Secretary—Treasurer.

Aiyar & Co.
Chartered Accountants.

MINUTES OF FOURTH ANNUAL GENERAL MEETING HELD ON 4th JANUARY, 1951 AT 4-30 P. M. THE INDIAN SCIENCE CONGRESS, BANGALORE

The meeting was attended by eleven members and two visitors.

1. Dr. B. B. Mundkur, the President of the Society took the chair.

2. The following resolution was passed, all members standing :

"The Members of the Indian Phytopathological Society place on record their deep sense of sorrow on the sad demise of Dr. K. C. Mehta, who was a Charter Member of the Society and its Vice-President for 1950, and convey their heartfelt sympathy and condolence to the members of his family".

3. The minutes of meeting held on 3-1-1950 were read and confirmed.

4. The Secretary read his report for 1950. There was a discussion on the Secretary's proposal to raise the subscription. A majority of members felt that efforts should first be made to reduce the cost of printing and to postpone the consideration of this proposal till next year. Subject to proper audit of accounts the report was adopted.

5. The ballot papers were opened by Dr. R. P. Asthana and Mr. D. D. Gupta and the following office bearers were declared elected for 1951 :

President	Dr. M. K. Patel
Vice-President	Dr. R. P. Asthana
Councillors	Dr. M. K. Hingorani
	Dr. P. R. Mehta
	Dr. S. R. Bose
	Dr. S. Vaheeduddin
	Dr. M. N. Kamat
	Sri T. S. Ramakrishnan

6. A vote of thanks to the Indian Council of Agricultural Research and to the National Institute of Science for subsidising the printing of INDIAN PHYTOPATHOLOGY was unanimously passed.

7. The meeting terminated after passing votes of thanks to the Chairman, the retiring office-bearers and to the Indian Institute of Science for affording the necessary facilities for holding the meeting.

R. PRASADA
Secretary-Treasurer

B. B. MUNDKUR
Chairman

INDIAN PHYTOPATHOLOGICAL SOCIETY

Instructions to Authors

Membership in the INDIAN PHYTOPATHOLOGICAL SOCIETY is prerequisite to publishing in INDIAN PHYTOPATHOLOGY but the Editorial Board may relax this rule in the case of contributions of exceptional merit and communicated with a special recommendation by a member. The Editorial Board may invite distinguished scientists to contribute articles of interest to the Society.

Contributions should be on one side of the page, double spaced, with a 1½th inch margin on the left. In form and style, such as punctuation, spelling and use of italics, the manuscript should conform to the best Journals in the U. K. and U. S. A. Authors should strive for a clear and concise style of writing. The name and address of the institution at which the work was done should be cited. Tables should be numbered and each table should have a heading stating briefly its contents. References to literature should be made as foot notes *only* when four or fewer citations are given. If there are more, they should be listed under 'REFERENCES' at the end of the paper and referred to by date in brackets in the body of the paper. Citations should give the name of the author (or authors), his (or their) initials, year of publication and the full title correctly, followed by the name of the Journal and volume and page numbers. If the title is in a foreign language, then the diacretic signs and capitalization should be as in the original. The names of the Journals should be as abbreviated in the WORLD LIST OF PERIODICALS, 2nd ed., 1934, but as that book may not be available to all, contributors are requested to give the titles in full. Abbreviating will, in that case, be done by the Editors. If an article has not been seen in original, then that should be clearly stated.

Because of high cost of half-tone blocks, carefully made line drawings on Bristol board in black ink will be preferred. Photographs when necessary should be printed on glossy contrast paper and be of best quality. Full page figures and photographs should be made to reduce to 4 x 6½ inches, the standard size for all plates. Each author is allowed one page of half-tone illustration for each article or its equivalent, and the cost of half-tone blocks and paper in excess will be charged to author. Drawings must be drawn to standard scales, so that they can be compared with one another, *e. g.* x 10, x 50, x 100, x 250, x 500 etc. It is not always possible to get a magnification at a round figure with a camera lucida but the printer can readily reduce drawings at any magnification to the standard, provided a scale is added to the drawing. The scale should measure from 5 to 10 cm. the longer the better and the printer should be instructed to reduce this line to the desired magnification.

Authors are invited to consult Bishby's 'An Introduction to Taxonomy and Nomenclature of Fungi' (1945), pp, 38-48 and Riker's 'The preparation of manuscripts for Phytopathology' Phytopathology 36 : 953--977, 1946, before preparing their mss. and figures.

Articles will be published in the order of their approval for publication but the address of the retiring President and invitation articles will be published when received.

To comply with the International Rules of Botanical Nomenclature, latin descriptions must be supplied to validate new species and genera.

Authors requiring reprints with or without covers should place an order for the copies wanted at the time of returning the proofs and they will be charged actual cost.

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